INTERNAL SECRETION OF THE GERMINAL TISSUE OF THE TESTES AND PROSTATIC HYPERTROPHY

BY

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INTERNAL SECRETION OF THE GERMINAL TISSUE OF THE TESTES AND PROSTATIC HYPERTROPHY

INAUGURAL DISSERTATION

BY

NILS TÖRNBLOM

MED. LIC., SMÅL.

By due permission of the most experienced Medical Faculty of the University of Upsala, to be publicly discussed in the Institute of Physiology, on May 2nd, 1942, at 10 o'clock a. m., for the degree of doctor of medicine

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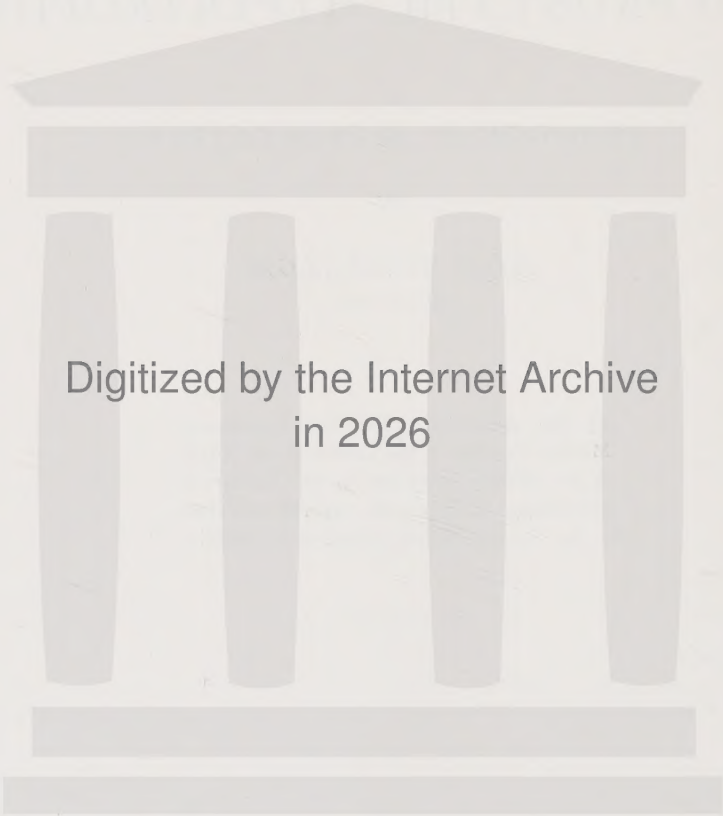
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Internal Secretion of the Germinal Tissue of the Testes and Prostatic Hypertrophy.

By

NILS TÖRNBLOM.

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Preface.

The work submitted here was carried on at the Physiological Institute, Upsala.

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Upsala, March 1942.

Nils Törnblom.

Introduction.

In the discussion round the etiology of prostatic hypertrophy, the question of the internal secretion of the testes and the importance of the sex hormones has during recent years advanced more and more into the foreground. In spite of intensive research, none of the hypo-

theses set up as regards the etiology of prostatic hypertrophy could be proved, nor have any been generally accepted. An important cause of the problem's remaining unsolved seems to be that our knowledge of the internal secretion of the testes is still incomplete in spite of the great progress made during the two last decades. There is still no answer to so central a problem as to whether there is *one* hormone formed in the testes, or if — as in the ovaries — there are two. At present only one is known, testosterone. It is formed in the interstitial cells and regulates *inter alia* the growth of the accessory sex glands.

Certain investigators (MOTTRAM and CRAMER, MARTINS, MCCULLAGH) have earlier assumed that another hormone, with effect principally on the hypophysis, is formed in the germinal tissue. This hormone is thought to inhibit the hypophysis' production of gonadotropic hormone.

After the preparation of pure testosterone, it has been found that this hormone not only affects the accessory sex glands but that it in addition has the action on the hypophysis which the investigators named above have wished to attribute to the postulated hormone of the germinal tissue. The administration of testosterone causes not only the castration changes in the accessories to disappear, but also those in the hypophysis. On this account, the assumption of a further testis hormone with special action on the hypophysis has been regarded as less probable, and the research in this field has stopped.

One of the hypotheses set up with regard to the etiology of prostatic hypertrophy is based on the assumption of a special testis hormone inhibiting the hypophysis. The internal secretion of the germinal tissue is thought to decrease with increasing age, and this releases via the hypophysis a heightened production of the interstitial cell hormone, testosterone. Prostatic hypertrophy would be due to an increased action of this hormone (NEMENOW, LOWER).

The aim of the investigation here presented has been

a) to determine if there is more than one hormone formed in the testes,
b) to determine if the germinal tissue of the testes has an independent endocrine function,
c) to determine if the cessation of any internal secretion in the germinal tissue sets up an increased formation of testosterone,
d) to isolate and characterize the hormone which may be formed in the germinal tissue of the testes,
e) to investigate whether increased action of testosterone induces changes in the prostate similar to those occurring in spontaneous prostatic hypertrophy.

HISTORICAL SURVEY.

Chapter 1. The Internal Secretion of the Testes.

General survey of the internal secretion of the testes.

The first proof that the testicles have a hormonal function was submitted by BERTHOLD in Göttingen in the year 1849, when he showed that the atrophy of the comb, which appears in cocks after castration, can be prevented by implantation of testes.

Numerous attempts have been made to substitute the cessation of the internal secretion of the testicles by injection with testis extract (ANCEL and BOUIN 1906, PÉZARD 1911). The effect on the combs of cocks was used as an indicator of the biological activity.[1] Somewhat later it was shown that an extract of male urine has the same effect (LOEWE and VOSS 1929, FRANK and HARROW 1929). The active substances in male urine, androsterone and dehydroandrosterone, were prepared in crystalline form by BUTENANDT and co-workers in 1931, 1932 and 1934.

These testicle and urine extracts are also active on mammals and prevent the development of the atrophic changes appearing in the accessory sex glands after castration (MOORE, GALLAGHER and KOCH 1929). A comparison between the action of testicle and of urine extract showed that the active ingredient in the former cannot be identical with the substances isolated from urine (LAQUEUR and co-workers 1931, 1935). Thus the testicle extract has a considerably greater effect on the accessory sex glands of mammals than urine extract with the same effect on the cock's comb. In 1935, DAVID and co-workers isolated an androgenous substance, testosterone, from the testes of bulls. Testosterone is the most active of the androgenic substances and is regarded as the hormone proper. The greater part of the androgenic substances in urine are probably waste products which are formed at the breaking down of the testosterone and which are eliminated from the body via the kidneys. Dehydroandrosterone possibly comes from the cortex of the suprarenal capsules (CALLOW 1938).

Androsterone was synthesized in 1934, testosterone in 1935 (RUZICKA and co-workers 1934, 1935, BUTENANDT and HANISCH 1935).

[1] In the following account, androgens are used to designate comb-growth promoting substances.

Most authors consider testosterone to be formed in the interstitial cells of the testicles (Ancel and Bouin 1923, Bouin 1931). In cryptorchism and after X-ray treatment, the germinal tissue is atrophied to a high degree, the interstitial tissue being well preserved. The testicles still have hormonal activity.

Estrogen is also present in the male organism (Fellner 1913, Laqueur 1926). Normal men excrete considerable quantities of estrogens in the urine (Laqueur and co-workers 1927). The administration of estrogens affects the accessory sex glands of castrate males (Freud 1933, David, Freud and de Jongh 1934). This effect does not tally with that of the androgens, which restore these organs to their normal conditions. Estrogen affects smooth musculature and connective tissue, but does not restitute the cytological changes in the secretory cells.

It is not known whether the estrogens normally appear in sufficient quantity in the blood to have any influence on the male organism. Nor is it known whence they originate. Directly or indirectly, the testes must — at any rate in certain animals — be of importance to the production of these substances. It is possible that the larger part of the estrogens in the urine are, like the androgens, to be regarded as products of the breaking down of the testosterone. There is no doubt that estrogens are formed in other organs than the testes: some of them possibly come from the food eaten (Eng 1937, Koch 1937, Parkes 1937, Callow 1938, Moore 1939).

Normally, the testicles are controlled by the hypophysis. After hypophysectomy, atrophy of the germinal and interstitial tissues of the testicles sets in, their internal secretions cease, and as a result of this the accessories also atrophy (Smith and co-workers 1927, 1930, 1934). The cessation of the internal secretion of the hypophysis can be compensated by injecting with hypophysis extracts. It seems as if at least two different gonadotropic hormones are formed in the male hypophysis (Greep 1937). They do not seem be sexually specific, but tally with those appearing in the female organism. With the one, the follicle-stimulating hormone, it is possible to do away with the atrophic changes in the germinal tissue attendant on hypophysectomy, without at the same time affecting interstitial cells and accessories. The other, the luteinizing hormone, affects the interstitial cells, and also the accessories via an increase of the internal secretion of the testicles. It has only a slight effect on the germinal tissue (Greep, Fevold and Hisaw 1936). The effect on the germinal tissue possibly proceeds via increased formation of testosterone, which also affects the atrophy of the germinal epithelium occurring after hypophysectomy (Walsh, Cuyler and McCullagh 1934, Nelson and Gallagher 1936).

After the difference in the reaction of the testicles to the administration of follicle-stimulating and luteinizing hormones has been shown,

there seems to be hardly any doubt that testosterone, at any rate in mammals, is formed in the interstitial cells of the testes.

Just as hypophysectomy sets up changes in the testicles, so after castration do certain changes appear in the hypophysis, principally an increased formation of gonadotropic hormone (Evans and Simpson 1929, Martins 1929). This reaction is looked upon as compensatory (Moore 1937). The castration changes in the hypophysis can be prevented by administering testis extract.

The administration of estrogen to non-castrate males causes atrophy of the testicles and accessory sex glands, due to the fact that this hormone inhibits the hypophysis' production of gonadotropic hormone (Moore and Price 1930, 1932).

Internal secretion of the germinal tissue of the testes.

The idea of an independent endocrine function of the germinal tissue of the testes was advanced by Mottram and Cramer in 1923. They investigated the effect of radium treatment on rat testicles and, like earlier investigators (Albers-Schönberg 1903 and Bergonie and Tribondeau 1904—1905, Regaud and co-workers 1907—1909), were able to establish that the germinal epithelium becomes atrophied, but not the interstitial cells. At the same time they found that a histological examination of the anterior lobe of the hypophysis revealed similar changes to those following castration. Since the latter changes do not only appear after a total removal of the testicles but also in atrophy of the germinal epithelium, they felt justified in assuming that they might be caused by a cessation of the internal secretion of this last.

Other observations, too, taken to support the hypothesis that the germinal tissue of the testicles has an independent internal secretion, refer to the fact that such injury as causes atrophy of the germinal but not of the interstitial tissues sets up castration changes in the anterior lobe of the hypophysis. Since the secondary sex characters are not, as is the case after castration, obviously affected, the conclusion that the testicles produce two hormones has been suggested: one formed in the interstitial cells and affecting the secondary sex characters, the other formed in the germinal tissue and affecting the hypophysis (Martins 1930, Martins and Rocha 1930, 1931).

Atrophy of the germinal tissue can be induced by experimental cryptorchism, by ligation of the efferent ducts or internal spermatic artery and also, by treating the testicles with X-ray or radium. Evans and Simpson found in 1929 that the hypophysis of animals with experimental cryptorchism contains an increased amount of gonadotropic hormone, at the same time as it is enlarged and exhibits so-called castration cells. By experimental parabiosis in which they set up choelio-

anastomosis between males with experimental cryptorchism and normal infantile females, MARTINS (1930) and MARTINS and ROCHA (1931) showed that the hypophysis produces an increased quantity of gonadotropic hormone in experimental cryptorchism. They report prostate and seminal vesicles to be normal. CUTULY and CUTULY (1937) have corroborated MARTINS' parabiosis experiments. They used an improved technique in that they took hypophysectomized females as partners.

MARTINS (1930, 1933) and MELLO (1933) likewise found, after ligation of the internal spermatic arteries or vasoligation, atrophy of the germinal epithelium, castration changes in the hypophysis and normal accessory sex glands.

The observation made by MOTTRAM and CRAMER as to hypophysis changes after radium treatment to the testes has already been touched on. They do not say whether the treatment had any effect on the size of the accessory sex glands or on other secondary sex characters. The effect of X-ray treatment has been studied more closely by WITSCHI, LEVINE and HILL (1932) and DESCLIN (1934); in principle, the observations they made as to the changes appearing in atrophy of the germinal epithelium are the same as were made earlier in experimental cryptorchism.

To support the hypothesis that two separate hormones are formed in the testicles, certain substitution experiments have in addition been submitted. These were made at a time when there was still no access to chemically pure sex hormones, so that the effects investigated were those partly of testicle extract or male urine extract, which were produced according to the principles laid down by MCGEE (1927), GALLAGHER and KOCH (1929) and others; partly simple aqueous extracts of testicles. The extracts according to MCGEE was produced by means of lipoid solvents and tested for their comb growth promoting action. When injected into castrate mammals, they induce, as said above, a complete restitution of the accessory sex glands.

In their attempts to substitute the castration changes in the hypophysis by means of testicle extracts, MARTINS (1929), MARTINS and ROCHA (1930 and 1931), MCCULLAGH (1932) and MCCULLAGH and WALSH (1934) found that injections of aqueous extract of testicle cause the castration changes in the hypophysis to disappear, although these extracts had no or only a negligible effect on the castration changes in the accessory sex glands. They affected not only the histological changes in the hypophysis but also the increased formation of gonadotropic hormone. The effect remained even when the extract had been treated with lipoid solvent (MCCULLAGH and WALSH 1935). The administration of lipoid extract with action on the castration changes in the accessory sex glands does not, according to MCCULLAGH (1932), MCCULLAGH and WALSH (1934) affect the hypophysis changes.

These experiments were considered to argue forcibly for the existence

of a testicle hormone, inhibiting the hypophysis and soluble in water, which was not identical with the lipoid-soluble hormone known earlier. McCullagh (1932) calls this new hormone »inhibin», on account of its effect on the hypophysis. The agreement between these substitution tests and the investigations into the changes occurring in experimentally induced atrophy of the testicular germinal tissue led to the supposition that »inhibin» is formed in the germinal epithelium.

The existence of a substance in the testicles which inhibits the hypophysis and is not soluble in lipoid, has also been supported by experiments with non-castrate animals. Myers, Vidgoff and Hunter (1933), and Myers, Vidgoff, Schade and Hunter (1936) fed noncastrate male rats with testicle substance which had been treated with lipoid solvent, and observed atrophy of the testicles and accessories. McCullagh and Walsh (1935), Vidgoff and co-workers (1938, 1939, 1940) have observed the same effect after injection of fat-free testicle extracts. McCullagh and Walsh (1935), McCullagh and Schneider (1940) report that such extracts also inhibit estrus in non-castrate females. The effect is supposed to be attained by »inhibin» inhibiting the hypophysis's production of gonadotropic hormone.

These investigations concerning a further testicle hormone have been exposed to severe criticism.

With regard to the changes appearing in experimentally induced atrophy of the germinal tissue, Nelson (1934, 1937) in a more detailed investigation of animals with experimental cryptorchism, has found that changes do not only occur in the hypophysis but that accessory sex glands are also different: they are smaller than in normal animals. The fact that castration changes occur not only in the hypophysis but also in the prostate and seminal vesicles means that the previously mentioned investigations into the changes attendant on experimentally induced atrophy of the germinal epithelium cannot be used to support the hypothesis that there is a further testicle hormone. The fact that castration changes are more marked in the hypophysis can be explained by saying that the hypophysis is more sensitive than the accessory sex organs to a lowering of the content of male sex hormone in the blood. Nor is it necessary to refer the lowered internal secretion of the testicles to atrophy of the germinal epithelium. It may be due to a lowered hormonal function of the remaining interstitial tissue.

After isolation of androsterone and testosterone, the postcontrol of the above-mentioned substitution experiments has been made. Here it was shown that if either of these substances are administered to castrate animals in sufficiently large quantities, not only are the castration changes in the accessory sex glands restituted, but also those in the hypophysis (Schoeller, Dohrn and Hohlweg 1936, Allanson 1937,

Hertz and Meyer 1937, Wolfe and Hamilton 1937, Cutuly and Cutuly 1938, Hamilton and Wolfe 1938, Biddulph 1939). It has been found that estrogen also restores the castration changes in the hypophysis in males (Meyer, Leonard, Hisaw and Martin 1932, Nelson 1934, 1935, Frank and Salmon 1936, Meyer and Hertz 1937, Schoeller, Dohrn and Hohlweg 1936, Wolfe and Hamilton 1937, Bunster and Meyer 1938, Cutuly and Cutuly 1938).

Nelson and Gallagher (1935) were unable to corroborate the observation of Martins and McCullagh concerning the effect of aqueous testicular extracts. Rubin (1941) found that feeding normal rats with bull testicle caused no change in their testicles or accessory sex glands. On the other hand, injection with lipoid-free aqueous testicular extracts caused a reduction in size of the latter organs. The effect is nevertheless ascribed to influence on the animals' state of nutrition, since they lost weight. The same effect was also obtained with extracts of other organs.

A possible explanation to the conflicting results as to the effect of aqueous testicular extracts is that they probably contained estrogen. As has been said above, this hormone affects the hypophysis in males. The amount of estrogen in the extracts have probably been very different, since the amount in the testicles varies very much from species to species (Eng 1937). Other circumstances may also have played a part. Estrogen appears in the organism partly in a free state, partly combined with glycuronic acid or other acids (Marrian 1933, 1938). In the latter form it is soluble in water and does not dissolve in lipoid solvents which do not mix with water. The biological effect of combined estrogen is slight in parenteral administration. It is greater in peroral administration, since the combined estrogen is hydrolyzed in the gastro-intestinal tract.

Since it has been proved that testosterone not only affects the castration changes in the accessory sex glands but also, if it is administered in sufficient quanties, those in the hypophysis, the assumption of a special testicle hormone inhibiting the hypophysis has been considered inprobable. There are no observations which do not fit in with the idea that only one hormone is formed in the testicles (Moore 1935, 1937, 1939). On the other hand, nor are there any observations which exclude the possibility of another testicle hormone being formed in the germinal tissue.

Chapter 2. Hormonal Theories as to the Etiology of Prostatic Hypertrophy.

Increased action of estrogen.

At present there are two hormonal theories as to the etiology of prostatic hypertrophy.

The one is based on the fact that the male organism contains both androgens and estrogens. Hypertrophy of the prostate would then be caused by the relation between the amount of androgens and estrogens being upset. In the ageing man the estrogens are thought to predominate, which leads to changes in the prostate gland.

This hypothesis is based on certain experimental observations. While he was investigating the carcinogenic action of certain sterols in 1933, LACASSAGNE found that when castrate male mice were given estrone over a rather longer period of time, it set up in them enlargement of the prostate with retention of urine and hydronephrosis. About the same time, but quite independently of this, the same observations were made by DE JONGH (1933) and BURROWS and KENNAWAY (1934). Histological examination shows hyperplasia of connective and muscular elements in the prostate. The epithelium is changed into stratified flat epithelium, displaying great similarities with the cornified epithelium of the vagina during estrus. Similar changes also occur in the seminal vesicles and other organs.

These changes disappear in part after the administration of androgens (DAVID, FREUD and DE JONGH 1934, DE JONGH 1935, ZUCKERMAN and PARKES 1936 and HARSH, OVERHOLSER and WELLS 1939). The epithelium becomes normal again. The hyperplasia of connective and muscular elements seems, on the other hand, to remain (RUSCH 1937).

The administration of rather larger quantities of estrogen may also set up the changes mentioned in non-castrate males, if they are given enough (DE JONGH 1935, WELLER, OVERHOLSER and NELSON 1936). The changes can then be removed by treatment not only with androgens but also with gonadotropic hormone, whose effect is probably due to the testicles being stimulated to an increased production of testosterone (DE JONGH and KOK 1936).

Changes similar in principle also occur in monkeys and dogs, and have been studied in detail in these animals by PARKES and ZUCKERMAN (1935), ZUCKERMAN and PARKES (1936), ZUCKERMAN and GROOME (1937), DE JONGH, KOK and VAN DER WOERD (1938), GAARENSTROOM, DE JONGH and KOK (1940).

The fact that it is possible by means of estrogens to produce changes displaying a certain likeness to those in spontaneous prostatic hyper-

trophy, and also that these changes are counteracted by androgens, has led to the assumption that human prostatic hypertrophy is caused by an increased action of estrogens. This increased action is set up by a change of the relation between estrogens and androgens. The production of the latter is presumed to diminish with increasing age; as the the estrogens are not thought to show a corresponding change, they would predominate and the condition for the mentioned change in the prostate would be there (LAQUER 1934, DE JONGH 1935, BURROWS 1935 and ZUCKERMAN 1936, GEISSENDÖRFER 1940).

Going on the favourable effect of androgens on the estrogen changes in the prostate, patients with prostatic hypertrophy have to a large extent been treated with testosterone-propionate or similar preparations. The results submitted vary. Many authors say that in a large number of cases there is a marked improvement (VAN CAPPELEN 1933, POLLAK 1938, MOORE and MCLELLAN 1938, ZEHN 1940, ALTENBURGER 1940). Other authors affirm quite as definitely that the treatment is valueless (THOMPSON and HECKEL 1939, CREEVY and REA 1940 etc.). Several cases have been observed of acute retention of urine directly connected with the treatment. It seems as though the results of these therapeutic experiments cannot be used to argue either for or against the hypothesis given as regards the etiology of the hypertrophy of the prostate.

In their urine, men excrete both androgens and estrogens. Many investigations have been made to find out whether the hormone excretion changes as the man gets older, or when there is prostatic hypertrophy. No particularly great changes seem to occur. Most of the investigators report that the quantity of androgens is moderately lowered, or else normal (DINGEMANSE, BORCHARDT and LAQUEUR 1937, RUSCH and KUNDERT 1937, MOORE, MILLER and MCLELLAN 1940, DE LAAT 1941). Only LOWER (1933) found an increase. As regards the relation of estrogens — androgens, ratio E : A, the reports vary more. According to RUSCH and KUNDERT (1937) it is increased in patients with hypertrophy of the prostate; according to HAMILTON, DEMING and ALLEN (1936) and DINGEMANSE and LAQUEUR (1940) it is lowered.

Apart from the varying results, it seems to be out of the question to deduce more subtle changes in the hormone production of the testicles from the amount of androgens in the urine. A detailed criticism of such a proceeding is outside the scope of this work. The problem is handled in papers by PARKES (1937), CALLOW (1938) and DINGEMANSE and LAQUEUR (1940). In this connection, it is an interesting fact that the administration of testosterone not only leads to an enhanced excretion of androgens, but also, and to a more or less equal degree, increases the quantity of estrogens (STEINACH and KUN 1937, HOSKINS, COFFMAN, KOCH and KENYON 1939, DORFMAN and HAMIL-

TON 1939). Also of interest is the following pronouncement by EVANS (1939): "Any superficial theory which connects androgen excretion with male sexual function breaks down completely when it is shown that men cannot be distinguished from women or normal men from eunuchs in respect of urinary androgen."

Increased action of androgen.

The other hormonal theory regarding the etiology of prostatic hypertrophy is diametrically opposed to the one above. According to this hypothesis, the hypertrophy is due to an increased effect of androgens. It is based on the assumption that a special hormone, inhibiting the hypophysis, is formed in the germinal tissue of the testicles. As the subject gets older, the spermatogenetic function of the testicles decreases and also, the production of this hypophysis-inhibiting testicle hormone. This releases an enhanced gonadotropic function of the anterior lobe. The gonadotropic hormone acts on the interstitial cells and raises the testosterone production of the testicles. The increased quantity of testosterone produces hypertrophy of the prostate (LOWER and co-workers 1932, 1933, 1934, 1935, 1936, 1940, MCCULLAGH 1932). The illness is thus thought to be caused by an over-function of the interstitial cells.

The same idea was put forward by NEMENOW as early as 1921, before the question of an independent hormonal function in the germinal epithelium had begun to be discussed.

In support of this hypothesis, a series of mainly experimental observations have been submitted. Castration is followed by atrophy of the prostate (HUNTER 1786). Administration of testosterone to dogs seems, according to RÖSSLE and ZAHLER (1938), to set up hyperplasia of the prostate. A heightening of the testicles' internal secretion by increased action of gonadotropic hormone stimulates the growth of the prostate, for this organ is enlarged in cases of testis tumours which are attended by increased prolan content in the urine; a histological investigation shows a diffuse hyperplasia of the glandular elements (HEIDRICH, FELS and MATHIAS 1930, FERGUSON 1933). Further, the administration of gonadotropic hormone to male monkeys causes enlargement of the prostate, accompanied by obstruction of the urethra (GESCHICKTER 1937). POWELL (1939) treated a case of cryptorchism in a 15-year old boy with gonadotropic hormone over a rather longer period. During treatment a strong prostatic enlargement set in, with difficulty in passing water and residual urine. The enlargement subsided when treatment ceased.

One has certain criteria for supposing that the gonadotropic function of the hypophysis is enhanced in older men. In women, an

over-function of the hypophysis with increased gonadotropic hormone in the urine sets in at the climacteric, when the internal secretion of the ovaries ceases. When examining urine from older men, Österreicher (1934), Kukos (1934), Saetre (1935), Stefanini (1937), Stimpfl (1940) and others similarly found an increased excretion of this hormone in a relatively large number of cases. The degree of increase is not, however, so high as in women. Similar observations have been made with regard to the quantity of gonadotropic hormone in hypophyses from older men and women (Witschi and Riley 1940). We are probably warranted in supposing the increase of gonadotropic hormone in the urine to be caused by a heightening of the hypophysis' gonadotropic function, due to the lowered internal secretion of the testicles. If we build on the idea that only one hormone (testosterone) is formed in the testicles, this fact would argue for a lowering of the production of this hormone in connection with increasing age. If, on the other hand, we accept the hypothesis that a hormone which inhibits the hypophysis is formed in the germinal tissue, this could be used to substantiate the prostatic hypertrophy's causation by an increased action of testosterone (Lower, Engel and McCullagh 1935). We would then say that the over-function of the hypophysis is conditioned by a lowered production of this inhibiting hormone. The increased content of gonadotropic hormone stimulates the interstitial cells to an enhanced formation of testosterone.

Heller, Heller and Severinghaus (1941) could not confirm the reports of a greater amount of gonadotropic hormone in urine from older men. The investigations into the excretion of androgens in the urine have already been reviewed.

Since the hypothetical testicle hormone »inhibin» has not been isolated, a peroral administration of testicle substance has had to be resorted to in therapeutic experiments. Improvement is reported in about 50 % of the cases (Lower, Engel and McCullagh 1935, Lower 1936, McComb and Pearse 1937).

Starting on a hypothesis of his own, which in principle differs little from those of Lower and McCullagh, Wugmeister (1937) has with good results treated prostatic hypertrophy patients with estrogen.

If we assume that prostatic hypertrophy is due to an increased action of androgens, the most rational hormonal therapy ought then to be castration. In the light of the favourable effect oophorectomy has on uterine myoma, Ramm in Christiania and White in Philadelphia (1893) used this on patients with hypertrophy of the prostate. The results were good. In a large number of cases, the operation was followed by rapid atrophy of the prostatic enlargement (White 1895, Cabot 1896). During the subsequent years, a large number of such operations were performed. The reports as to the value of the treatment vary (Fremantle 1897, White 1904, Thomson-Walker 1930).

This form of treatment has, since about 1900, practically disappeared. In veterino-medical practice it is still used with excellent results in prostatic hypertrophy of dogs (Hobday 1924). That this form of treatment has disappeared does not necessarily mean that it was ineffective. If we want to use the results of the operation as an argument in the etiology of prostatic hypertrophy, we must take into account that we do not know if, or how far, the changes in the prostate are reversible. That the prostate in such cases really is under the influence of androgen has been supported by a work of Huggins and Stevens (1940), whose patients displayed atrophy of the prostatic secretory epithelium after castration.

Both aspects of the etiology of prostatic hypertrophy are based on assumptions. It does not seem possible to decide which is the more likely. At endocrinology's present stage, it is possible to present almost any hypothesis as probable by advancing the pros and suppressing the cons.

AUTHOR'S INVESTIGATIONS.

Chapter 3. Attempts to Substitute the Cessation of the Internal Secretion of the Testes by Administering Testosterone.

Introduction. The assumption that the germinal tissue of the testicles has an independent internal secretion and that a hormone other than testosterone is formed there has been accepted as a working hypothesis.

No investigations have previously been made as to whether the amount of testosterone normally appearing in the blood is able to keep at bay the castration changes in the hypophysis. To find this out, young male rats were castrated and attempts made to compensate the cessation of the testicular internal secretion by administering testosterone in such quantity as to bring the testosterone content in the blood of the castrate animals rather over that in normal animals. The growth of the prostate and seminal vesicles were used as indicators of the testosterone content of the blood. Young animals were chosen on the score that they show a vigorous growth of the accessory sex glands. Concerning the castration changes, the features followed have been partly the increase in weight of the hypophysis, which sets in after castration, partly the appearance of so-called castration cells.

Methods and observations. The normal growth of the prostate and seminal vesicles in young white rats was determined by taking these organs from normal animals of different ages, and weighing them. The weight of the hypophyses was also determined. The number of normal animals was 72. A group of 12 animals was examined at a weight of about 30 gm. A second group, similarly of 12, was examined a week after attaining this weight, a third group after yet another week, and so on. The sixth group was thus examined 5 weeks after the animals had reached a weight of 30 gm. The weights of hypophyses, prostates and seminal vesicles are shown in fig. 1. Regarding the seminal vesicles, the mean weight of each animal's pair has been given.

The animals dosed with testosterone were castrated at a weight of 30 gm. They were examined 1, 2, 3, 4 and 5 weeks respectively after castration — at corresponding times to the normal animals. They were given testosterone once daily, from the day of castration inclusive.

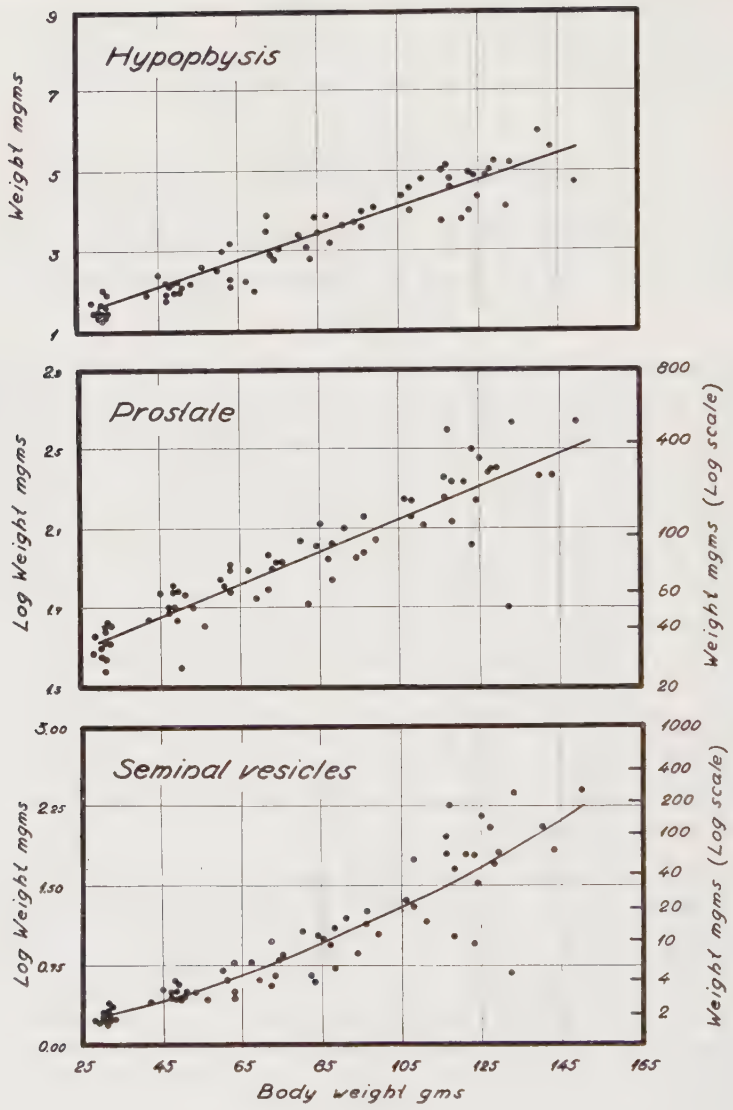


Fig. 1. Weight of hypophysis, prostate and seminal vesicles of normal rats according to body weight. • Observed weight. ——— Calculated weight. Formulas (1), (2) and (3).

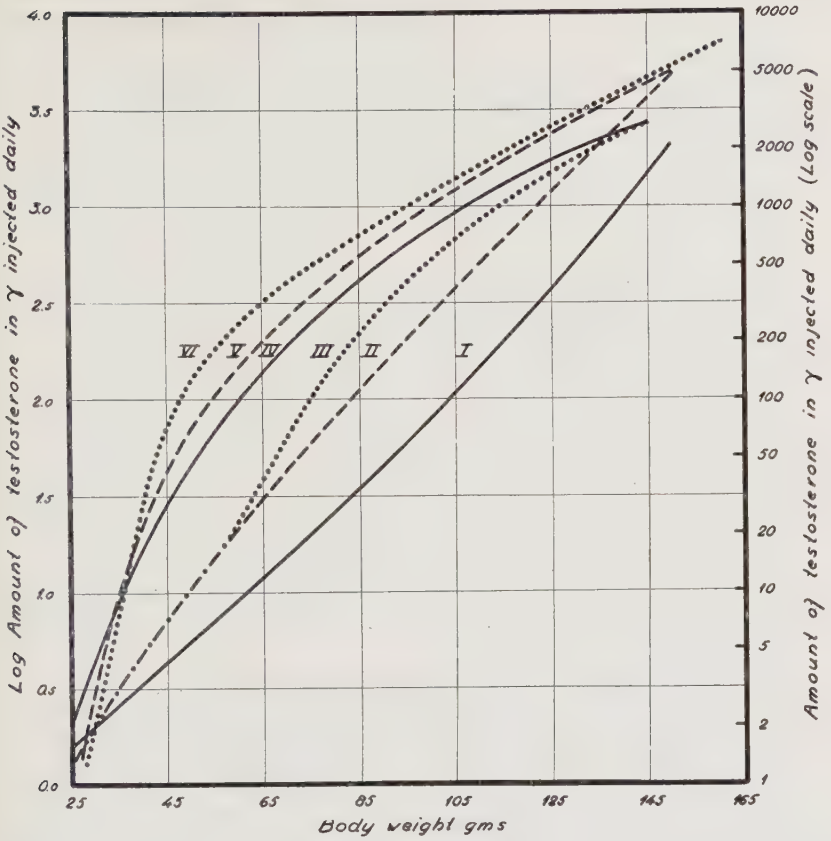


Fig. 2. Diagram of the amount of testosterone given the animals in Series I—VI. The lines give the amount of testosterone injected daily. Testosterone has been given according to body weight.

The amount given was determined by their weight, and is shown in fig. 2. The testosterone was dissolved in sesame oil and injected subcutaneously. The solution was prepared by dissolving crystalline testosterone in peroxide-free ethyl ether. The sesame oil was added to the ether solution, after which the ether was evaporated off by heating to 100° C in a vacuum. The concentrations used were 1 cc = 1, 1 $^1/_2$, 2, 3, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80, 100, 150, 200, 300, 400, 600, 800, 1,000, 1,500, 2,000 γ. At the concentrations 1.5 and 2 mg testosterone per cc sesame oil, crystals of testosterone were after a time precipitated in the solution, so that these solutions were prepared *ex tempore*. The oil solution was applied with a so-called tuberculin syringe graduated in 0.01 ccs. The amount injected each time varied between 0.085

and 0.125 ccs. Larger quantities were given of the concentration 1 cc=2 mg.

The first series of castrate animals were given testosterone according to line I in fig. 2. The intention was to administer testosterone in such quantity that the increase in weight of the accessory sex glands coincided with or was greater than the increase shown by normal animals. The weight of the prostate and of the seminal vesicles in the animals of this series (Series I) was less than in normal animals of corresponding weight.

The result in Series I was used as a basis for calculating the dose of testosterone to the next series (Series II). In this series, too, the growth curves for prostate and seminal vesicles were below those of the normal animals.

The growth of these organs at a dosage according to line III (fig. 2) is shown in fig. 3. The weight of prostate and seminal vesicles in the animals treated with testosterone tallies on the whole with that of the normal animals. This administration of testosterone does not seem to prevent the appearance of castration changes in the hypophyses, these latter organs being larger than in the normal animals.

When testosterone was given according to lines IV or V (fig. 2), the growth of prostate and seminal vesicles seems to be more than that in the normal animals (figs. 4 and 5). In spite of this, the animals' hypophyses were still enlarged.

A further increase in the testosterone dose, line VI (fig. 2), caused an increase in growth of the prostate and seminal vesicles. It also caused the enlargement of the hypophysis to disappear (fig. 6).

The hypophyses from a number of animals belonging to the series IV and V were examined histologically. They were fixed in a neutral formol-potassium bichromate solution according to Cowdry (1916), and stained with anilin blue and orange G according to Reese and McQueen-Williams (1932). The appearance of hypophyses from normal and castrate animals in the examination using this histological technique has been described in detail by Addison (1917), and also by Reese and McQueen-Williams. The most easily discernible histological castration change is the presence of so-called signet-ring or castration cells (figs. 56 and 57). 2—3 weeks after castration, these cells appear in abundance in the hypophyses from young male rats (Schoeller, Dohrn and Hohlweg 1936).

The hypophyses examined came from animals which had been killed 3, 4 and 5 weeks after castration. The result of an examination of these organs will be found in figs. 3 and 4. Each hypophysis examined corresponds to a circle, which has been placed in the diagram according to the weight of the animal when killed. Regard has only been paid to the presence of signet-ring cells. The absence of any such is indicated with ○. If the signet-ring cells were many, this is indicated with ●,

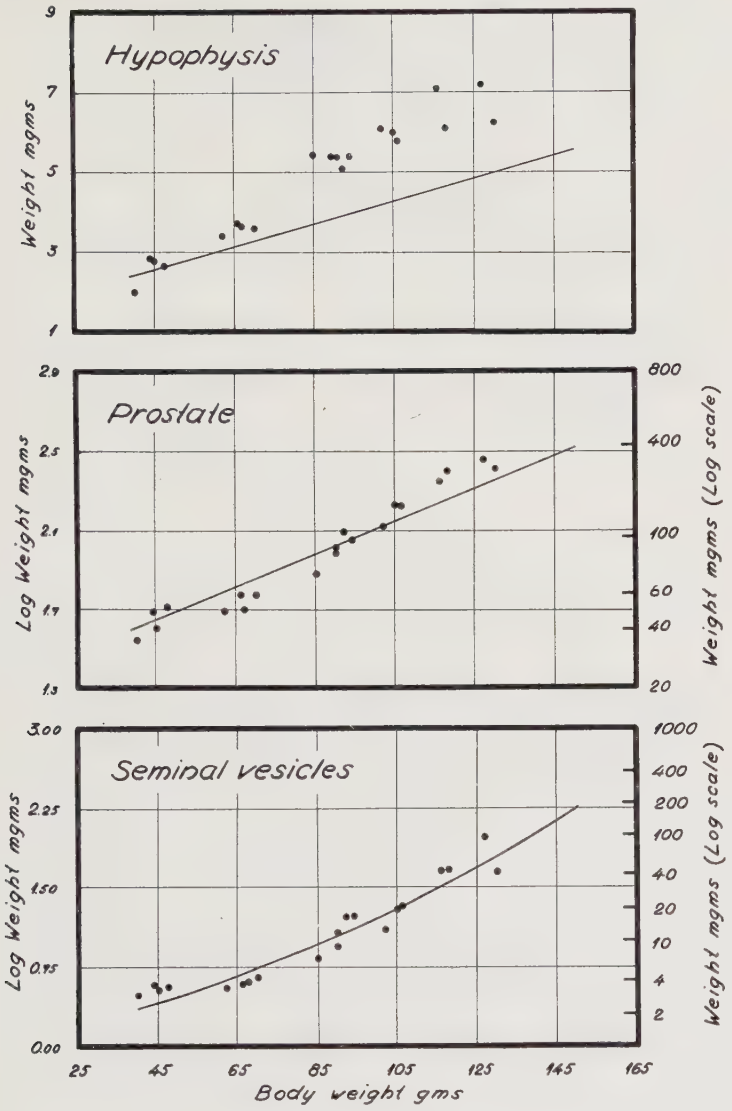


Fig. 3. Series III. Weight of hypophysis, prostate and seminal vesicles of castrate rats injected with testosterone according to III fig. 2. • Observed weight. ——— Calculated normal weight. Formulas (1), (2) and (3).

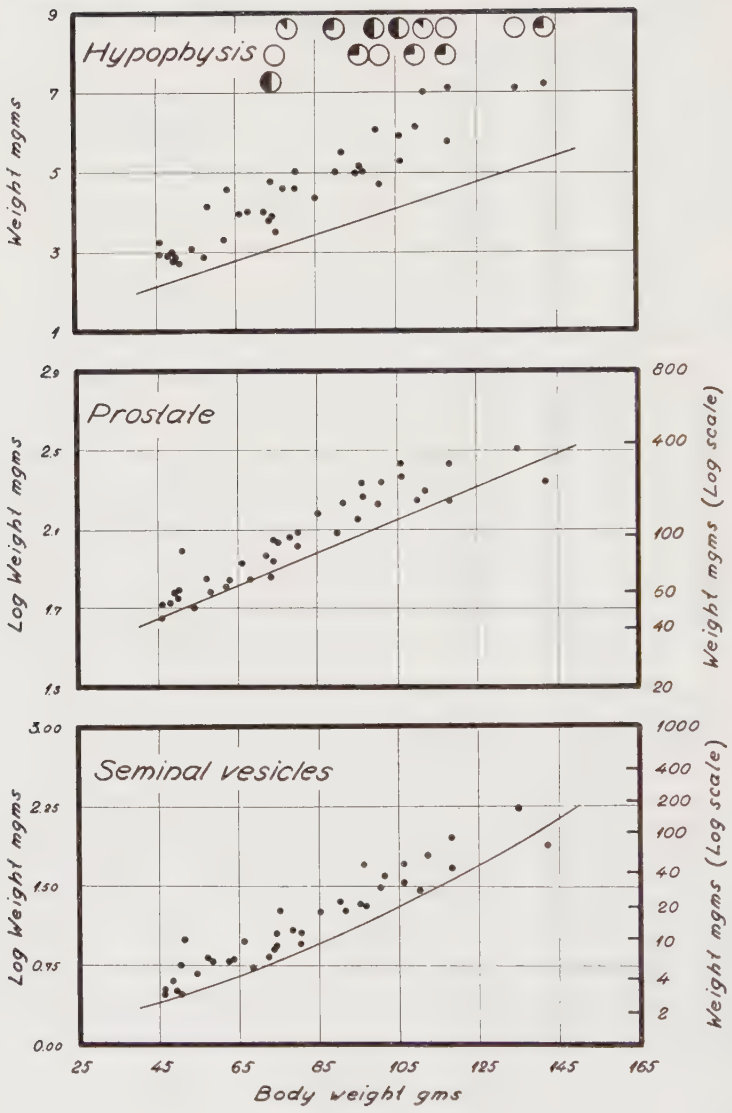


Fig. 4. Series IV. Weight of hypophysis, prostate and seminal vesicles of castrate rats injected with testosterone according to IV fig. 2. • Observed weight. ——— Calculated normal weight. Formulas (1), (2) and (3). The circles, ○, in the hypophysis-chart refers to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

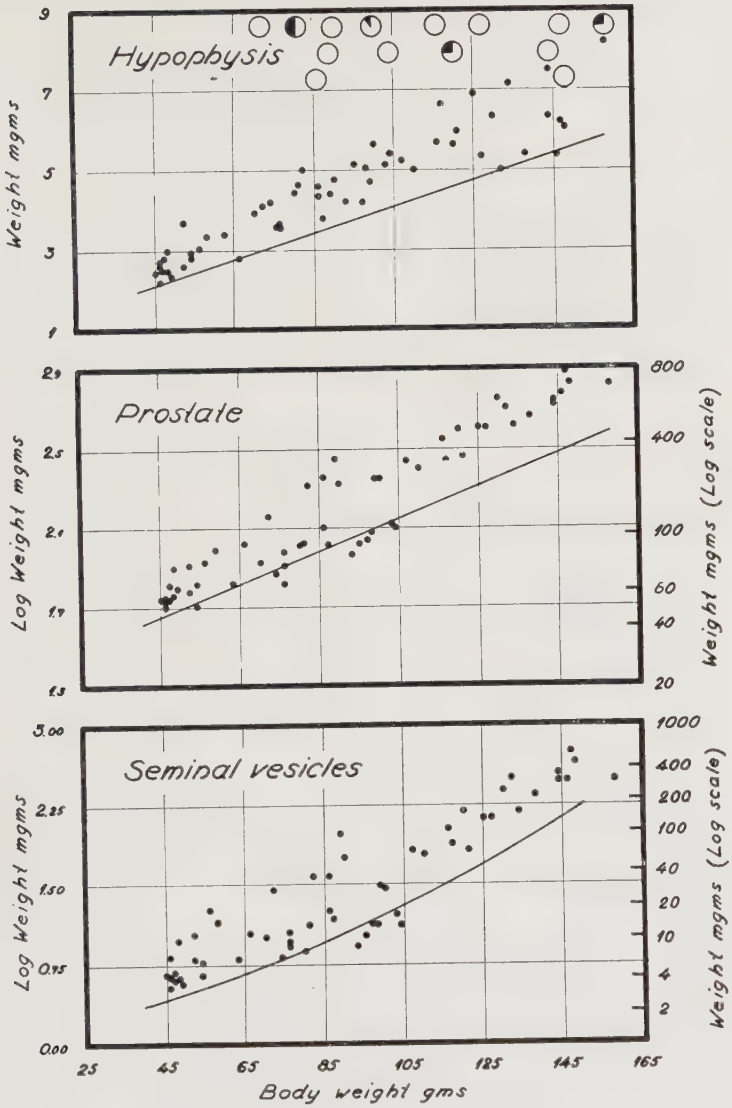


Fig. 5. Series V. Weight of hypophysis, prostate and seminal vesicles of castrate rats injected with testosterone according to V fig. 2. • Observed weight. ——— Calculated normal weight. Formulas (1), (2) and (3). The circles, ○, in the hypophysis-chart refers to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

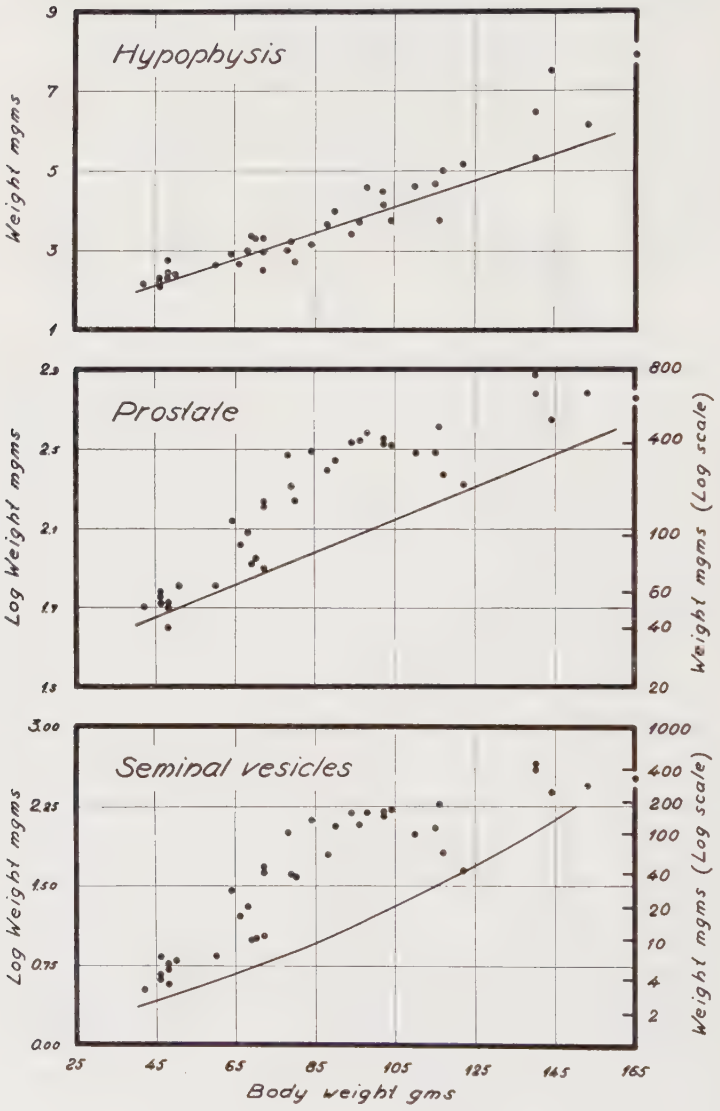


Fig .6. Series VI. Weight of hypophysis, prostate and seminal vesicles of castrate rats injected with testosterone according to VI fig. 2. • Observed weight. ——— Calculated normal weight. Formulas (1), (2) and (3).

intermediate stages with ◔, ◔, ◐ and ◕. Fig. 3 shows that such cells were present in most of the hypophyses from the animals belonging to Series IV. Of the 14 examined hypophyses from the animals in Series V, only 4 displayed signet-ring cells. The hypophyses from the animals in this series were nevertheless still enlarged.

Statistics. The statistical treatment is intended to determine whether the differences between the weight of hypophysis, prostate and seminal vesicles in the animals from the series IV and V and in normal animals of corresponding body weight are statistically significant. The material in these two series has been made into one (fig. 7).

The relation between organ weight and body weight is shown by the following regression equations.

$$y = a_0 + a_1 \cdot x; \quad y = a_0 + a_1 \cdot x + a_2 \cdot x^2.$$

Normal animals.

Hypophysis. $y = + 0.630 + 0.0329 \cdot x$ (1)

y = hypophysis weight in mg. x = body weight in gm.

Prostate. $y = + 1.286 + 0.00825 \cdot x$ (2)

Seminal vesicles. $y = + 0.0405 + 0.00567 \cdot x + 0.0000607 \cdot x^2$ (3)

y = log organ weight in mg. x = body weight in gm.

Series IV + V.

Hypophysis. $y = + 0.902 + 0.0417 \cdot x$ (4)

Prostate. $y = + 1.273 + 0.00996 \cdot x$ (5)

Seminal vesicles. $y = - 0.340 + 0.0188 \cdot x + 0.00000123 \cdot x^2$ (6)

Hypophysis. The divergence (α) from the normal of the observed hypophysis weights in the animals in Series IV + V has been calculated. The normal weight has been calculated according to equation (1). $M\alpha = + 1.038$. $\varepsilon(M\alpha) = \pm 0.064$. In calculating the difference (D) between the hypophysis weight in the animals in Series IV + V and in the normal animals, the standard error in the normal material has been calculated according to the standard error of a_0 at the medium, equation (1), $\varepsilon(a_0') = \pm 0.057$. $D \pm \varepsilon(D) = + 1.038 \pm 0.085$. The difference is 12.2 times the standard error of the difference.

Prostate. Calculations as for hypophysis. Normal weight according to equation (2). The difference (D) refers to the log weight of the prostate. $D \pm \varepsilon(D) = + 0.135 \pm 0.0154$. The difference ($D$) between the

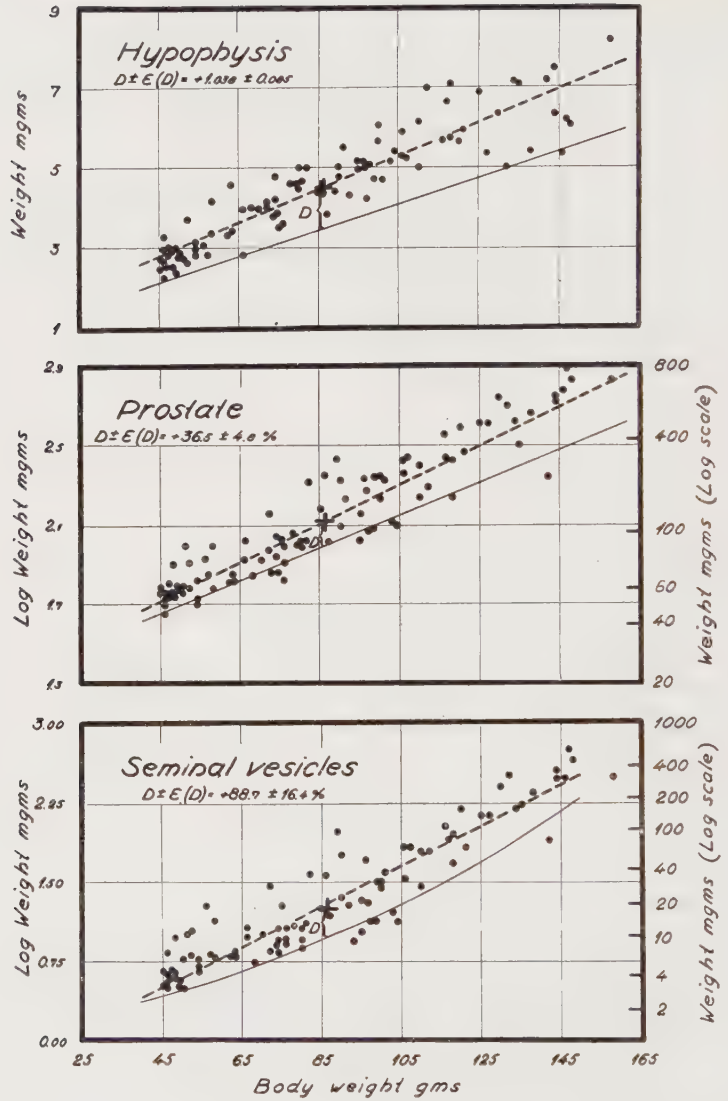


Fig. 7. Series IV + V. Weight of hypophysis, prostate and seminal vesicles of castrate rats injected with testosterone according to IV or V fig. 2. • Observed weight. — — — — Calculated weight. Formulas (4), (5) and (6). ——— Calculated normal weight. Formulas (1), (2) and (3). D = Difference between weight of hypophysis, prostate and seminal vesicles in the rats in Series IV + V and in normal rats. The difference is given in mgs for hypophysis, in per cent of normal weight for prostate and seminal vesicles.

weight of the prostate in the animals in Series IV + V and in the normal animals has been calculated in % of the normal weight. $D \pm \varepsilon(D) = + 36.5 \pm 4.8$ %. The difference is 7.6 times the standard error of the difference.

Seminal vesicles. Calculations as for prostate. Normal weight according to equation (3). The standard error in the normal material has been calculated according to the divergence from normal weight [equation (3)] of the separate observations. $D \pm \varepsilon(D) = + 88.7 \pm 16.4$ %. The difference is 5.4 times the standard error of the difference.

Thus, castration changes in the hypophysis cannot be prevented by administering to young castrate male rats testosterone in such quantity as to make the weight of the prostate and seminal vesicles somewhat higher than that of these organs in normal animals. The hypophyses in such castrates are enlarged.

Discussion. The growth of the prostate and seminal vesicles can only with reservations be used as an indicator of the blood's testosterone content. It is possible that the growth of these organs is not only determined by the testosterone content of the blood; other substances may also play a part. If castrates are given testosterone in such quantity that the growth of these organs tallies with that in normal animals, the testosterone content in the blood of the former animals may certainly have been greater than in that of the latter, but would not be less. When the amount of testosterone administered to castrates resulted in these organs growing more than those of normal animals, the testosterone content must have been greater. This hormone treatment is not able to prevent the increase in weight of the hypophysis, which appears after castration. If one assumes that testosterone is formed in the testicles, then there must also be another hormone formed there which, in relation to testosterone, is characterized by a stronger effect on the weight increase of the hypophysis compared with the effect on prostate and seminal vesicles.

Summary. Giving testosterone to young castrate male rats in such quantity that the weight of the prostate and seminal vesicles somewhat exceeds the weight of these organs in normal animals will not prevent the increase in weight set up in the hypophysis after castration. If it is assumed that testosterone is formed in the testicles, another hormone must also be formed there which, in relation to testosterone, is characterized by a stronger effect on the weight increase of the hypophysis compared with the effect on prostate and seminal vesicles.

Chapter 4. Changes in the Hypophysis and Accessory Sex Glands in Injuries to the Germinal Tissue of the Testes.

Introduction. A study has been made of the conditions of the hypophysis and the accessory sex glands in experimental cryptorchism, in ligation of the efferent ducts and after treating the testicles with X-ray; this study was based on the working hypothesis that two hormones are formed in the testicles, one in the germinal tissue and one in the interstitial cells (testosterone); and that the former hormone is characterized in relation to the latter by a greater action on the hypophysis compared with that on the accessory sex glands. By means of the methods given above, it is possible to induce a more or less high degree of atrophy of the germinal epithelium without corresponding injury to the interstitial tissue.

To be able to submit possible changes in proof of a hormonal function of the geminal tissue, it is necessary to show that they are not due to a lowered internal secretion of the interstitial cells.

The intention has been to investigate whether it is possible, by injuring the germinal tissue, to produce castration changes in the hypophysis without at the same time inhibiting the growth of the accessories.

In the attempts to compensate the cessation of the internal secretion of the testicles by administering testosterone, it was observed that giving this hormone to young castrate male rats in sufficient quantity to raise the weight of their prostate and seminal vesicles somewhat above that of these organs in normal animals was not able to prevent the weight increase in the hypophysis which sets in after castration. There is therefore reason for supposing that the postulated second testicle hormone affects this increase, and that stopped production of this hormone induces weight increase of the hypophysis.

Experimental cryptorchism.

Methods. Young male white rats were used as test animals. At the time of the operation they weighed about 30 gm. The abdominal cavity was opened and the testicles pulled out. Since the gubernaculum Hunteri connects the lower end of the testicle to the testicle sheaths, they will be turned inside out. The connecting tissue is cut (fig. 8). The testicles are fixed as high as possible in the abdominal cavity, by sewing fast the attached remains of their sheaths to the lateral wall. In this way their supply of vessels is not damaged. The internal

orifices of the inguinal canals are closed. 25 animals were operated. 9 animals were examined after 8 weeks, 8 after 12 and 8 after 16. Hypophysis, testicles, prostate and seminal vesicles were weighed.

The normal animals were similarly investigated 8, 12 and 16 weeks after reaching a weight of 30 gm.

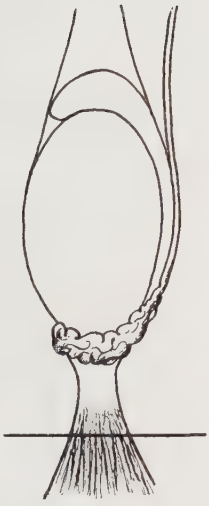


Fig. 8.

Observations and statistics. The weights of hypophysis, testicles, prostate and seminal vesicles are shown in figs. 9—11. As regards testicles and seminal vesicles, the weight refers to the mean of both in each animal.

The statistical treatment is intended to determine whether there is any significant difference between the weight of these organs in animals with experimental cryptorchism and in normal animals.

The relation between the weight of the organs in question and the body weight is shown by the following regression equations ($y=a_0+a_1 . x$).

Normal animals.

Hypophysis. $y=+0.523+0.00158 . x$ (7)
$a_1 \pm \varepsilon(a_1)=+0.00158 \pm 0.00034$

Prostate. $y=+2.279+0.00295 . x$ (8)
$a_1 \pm \varepsilon(a_1)=+0.00295 \pm 0.00047$

Seminal vesicles. $y=+2.197+0.00201 . x$ (9)
$a_1 \pm \varepsilon(a_1)=+0.00201 \pm 0.00091$

y=log organ weight in mg. x=body weight in gm.

Animals with experimental cryptorchism.

Hypophysis. $y=+0.535+0.00189 . x$ (10)
$a_1 \pm \varepsilon(a_1)=+0.00189 \pm 0.00030$

Prostate. $y=+2.464+0.00141 . x$ (11)
$a_1 \pm \varepsilon(a_1)=+0.00141 \pm 0.00084$

Seminal vesicles. $y=+2.159+0.00164 . x$ (12)
$a_1 \pm \varepsilon(a_1)=+0.00164 \pm 0.00102$

Comparison is easier if those regression lines to be compared are parallel. Since the difference with regard to a_1 in the regression equations to be compared never exceeds 1.6 times the standard error of the difference, equations have been calculated for the animals with experimental cryptorchism where a_1 is made equal to a_1 in the corresponding equations for normal animals (7), (8) and (9).

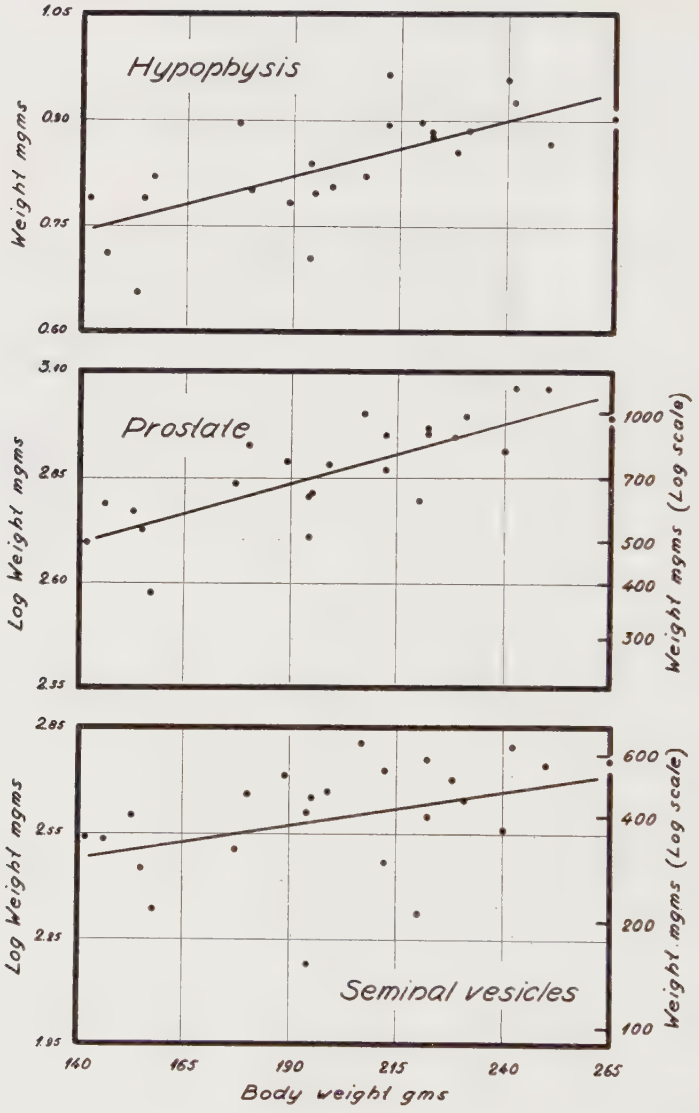


Fig. 9. Weight of hypophysis, prostate and seminal vesicles of normal rats according to body weight. • Observed weight. ——— Calculated weight. Formulas (7), (8) and (9).

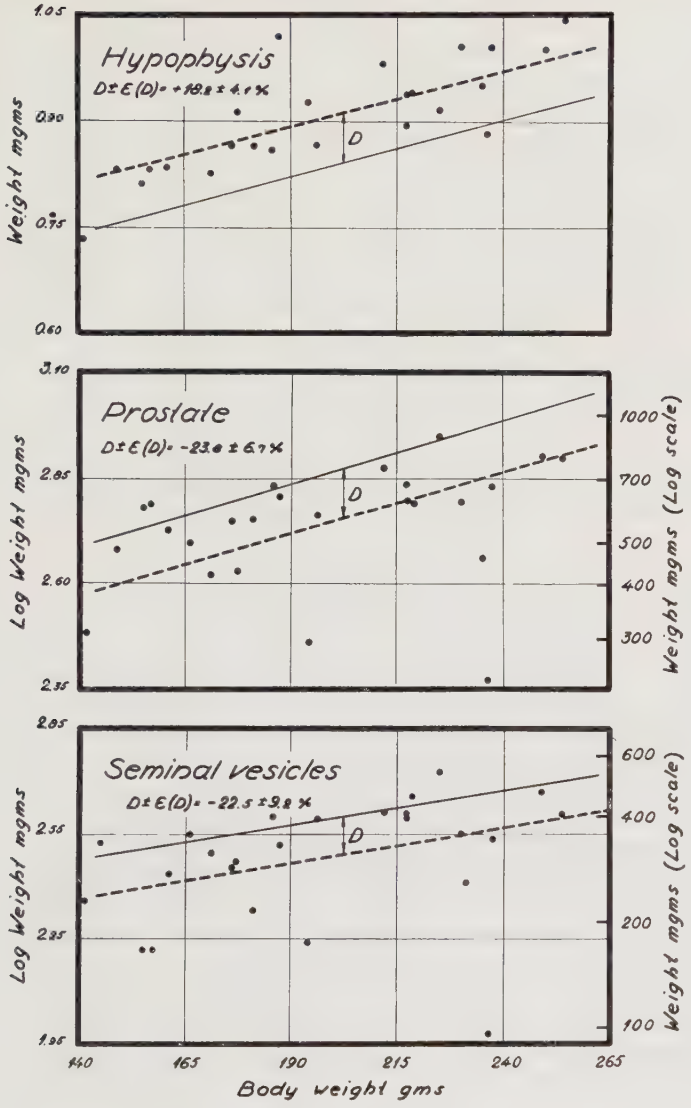


Fig. 10. Weight of hypophysis, prostate and seminal vesicles of cryptorchid rats according to body weight. • Observed weight. — — — — Calculated weight. Formulas (13), (14) and (15). ——— Calculated normal weight. Formulas (7), (8) and (9). D = Difference between weight of hypophysis, prostate and seminal vesicles in cryptorchid rats and in normal rats. The difference is given in per cent of normal weight.

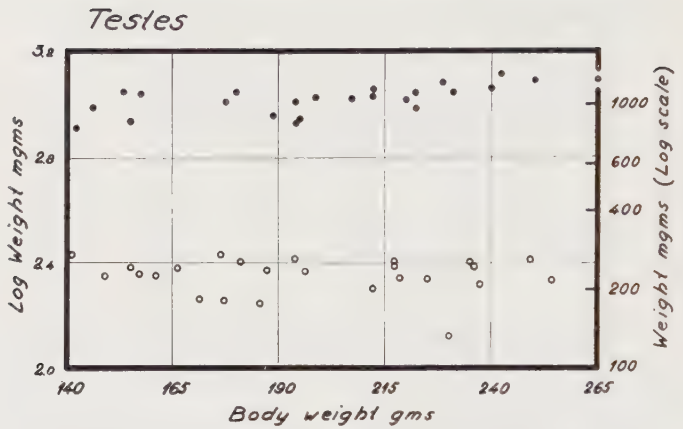


Fig. 11. Weight of testes of normal and cryptorchid rats according to body weight. • Observed weight, normal. o Observed weight, cryptorchid.

Hypophysis.	$y = +0.596 + 0.00158 . x$	(13)
Prostate.	$y = +2.160 + 0.00295 . x$	(14)
Seminal vesicles.	$y = +2.086 + 0.00201 . x$	(15)

Fig. 10 shows the difference in weight of hypophysis, prostate and seminal vesicles in animals with experimental cryptorchism and in normal animals of the same weight. The calculation has been made according to the principles given on pp. 23 and 25.

Discussion. Fig. 10 shows that the weight of the hypophysis in the animals with experimental cryptorchism is increased, as in castrate animals; the weight of the prostate and the seminal vesicles is lowered. The attempt to induce weight increase of the hypophysis without simultaneous inhibition of the accessory sex glands has thus not been successful with experimental cryptorchism.

Experiments have also been made with ligation of the ductus deferens and ductuli efferentes. No weight increase in the hypophysis was observed in so doing.

X-ray treatment of testicles.

Introduction. The X-rays affect not only the germinal tissue but also the interstitial cells. If, on the assumption that one hormone is formed in the germinal tissue and another in the interstitial cells, one wishes to study the changes which may set in on the cessation of

the former's internal secretion, the X-ray treatment should be performed in such a way as to damage the interstitial cells as little as possible.

According to Regaud (1922), Schinz and Slotopolsky (1925), Regaud and Ferroux (1927), Sartory, Sartory and Meyer (1931) and Ferroux, Regaud and Samssonow (1938), the difference in X-ray sensitivity between the germinal and interstitial tissues can be heightened by not giving the whole dose at once but dividing it up into a number of smaller ones which are given at intervals of some days or weeks (fractionating).

According to Kirchhoff and Kelbling (1937), an increase of the time for each X-ray dose (protraction) is of no importance, nor is the wave-length of the irradiation (Yamakawa 1927).

Using rabbits, Ferroux and Regaud (1927) have investigated the effect of ischæmia on the X-ray sensitivity of the germinal epithelium, and say that the latter is thereby protected. This nevertheless seems questionable.

In the investigations mentioned, the X-ray sensitivity has been estimated on the basis of the morphological changes occurring in the irradiated organ.

Methods, statistics and results. Voltage of X-ray tube 165 kW. Tube current 2 or 7 m. a. Filter 0.5 mm Cu + 1.0 mm Al. Voltage and current have been checked. The radiation intensity was measured by condensator chambers (Sievert 1934) and was submitted to constant control.

Young male white rats were used as test animals. They were X-rayed two or three times. At the first treatment they weighed about 30 gm. When the animals were treated twice, the time between the two treatments was two weeks; if three times, the interval between was one week. During the treatment, they were fixed on their backs. Those parts of the animal not irradiated were protected by lead sheeting 2 mm thick. During treatment, the testicles had been brought down into the scrotum. The lead sheet was so formed as to hold them in this position (fig. 12).

The animals were examined 5 weeks after the first X-ray treatment. Hypophysis, prostate, seminal vesicles and testicles were weighed. The normal animals were similarly examined 5 weeks after reaching a weight of 30 gm.

Further experimental conditions, and also the difference between the weight of hypophysis, prostate and seminal vesicles in X-rayed testicles and in those of normal animals, are shown in table 1, after p. 32, and fig. 13. The differences have been calculated according to the principles given in p. 23 and 25.

Unfortunately, not all the test animals come from the same breeding-stock, since the earlier one perished in a fire. The material was

complemented by animals from another breeding-stock. Series with animals from the first stock are distinguished with the letter A from the others having the letter B. A series have only been compared with A series, B series with B series.

The relation between organ weight and body weight is shown in the following regression equations ($y = a_0 + a_1 . x$).

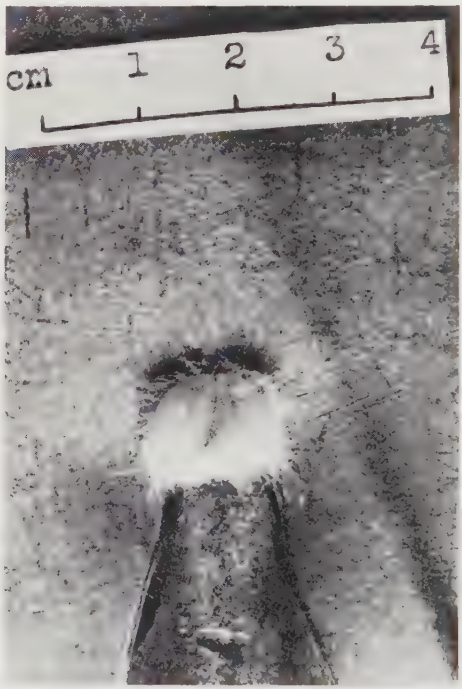


Fig. 12.

Normal animals, Series AI (figs. 14 and 22).

Hypophysis. $y = +0.301 + 0.00306 . x$ (16)
$a_1 \pm \varepsilon (a_1) = +0.00306 \pm 0.00037$

Prostate. $y = +1.953 + 0.00378 . x$ (17)
$a_1 \pm \varepsilon (a_1) = +0.00378 \pm 0.00061$

Seminal vesicles. $y = +1.057 + 0.00724 . x$ (18)
$a_1 \pm \varepsilon (a_1) = +0.00724 \pm 0.00115$

Testicles. $y = +2.756 + 0.00148 . x$ (19)
$a_1 \pm \varepsilon (a_1) = +0.00148 \pm 0.00030$

y = log organ weight in mg. x = body weight in gm.

Table 1. *Difference (D) between weight of hypophysis, prostate and seminal vesicles in rats with X-ray-treated testicles, and in normal animals (series A I, 51 animals, series B I, 20 animals). The difference is expressed in % of the normal weight. The sign + shows increase, the sign — decrease. D ± ε(D). The difference is given in italics if it is equal to or greater than 3 times the standard error of the difference.*

Total dose	Time of exposure in each X-ray treatment	10 mins.					15 mins.	20 mins.			40 mins.	
	Number of X-ray treatments	2				3	3	2		3	2	3
	Ratio of the doses	2 : 1				2 : 1 : 1	1 : 1 : 1	2 : 1		2 : 1 : 1	2 : 1	2 : 1 : 1
	Circulation	Normal	Stopped 15 mins.	Stopped 30 mins.	Stopped 60 mins.	Stopped 30 mins.	Stopped 30 mins.	Normal	Stopped 30 mins.	Stopped 30 mins.	Stopped 55 mins.	Stopped 55 mins.
87.5 r	Series No. of animals Hypophysis Prostate Seminal vesicles										AXI a 11 + 8.2± 3.6 % + 3.2±10.9 % + 8.6±21.6 %	AXII a 5 +14.8± 7.0 % +17.4±12.6 % +24.8±25.1 %
125 r	Series No. of animals Hypophysis Prostate Seminal vesicles										AXI b 17 + 6.5± 2.6 % + 9.3± 7.0 % +15.3±14.2 %	AXII b 5 + 8.0± 3.5 % +22.0±11.0 % +16.1±15.7 %
175 r	Series No. of animals Hypophysis Prostate Seminal vesicles						AVII a 8 + 5.3± 3.5 % −20.0± 7.3 % −26.7±11.4 %			AX a (fig. 19) 12 *+18.4± 3.2 %* + 5.3± 4.9 % + 6.2±11.2 %	AXI c 17 + 8.8± 3.1 % − 1.5±15.9 % − 5.5±10.3 %	
250 r	Series No. of animals Hypophysis Prostate Seminal vesicles		AIII a 8 + 0.9± 2.3 % −16.6± 7.0 % −37.7±15.0 %		AV a 7 − 1.4± 3.3 % *−32.0± 4.7 %* *−51.6±11.2 %*	AVI a 13 + 1.5± 3.2 % − 8.2± 5.0 % −26.8± 9.0 %	AVII b 20 *+13.6± 2.8 %* *−14.9± 4.5 %* *−23.1± 6.7 %*		AIX a (fig. 17) 17 *+13.5± 3.6 %* + 3.1± 6.5 % + 2.2±11.6 %	AX b (fig. 20) 22 *+15.3± 2.8 %* − 3.1± 5.9 % − 3.0±13.2 %	AXI d (fig. 21) 24 *+15.4± 2.6 %* − 0.5± 4.4 % − 4.1± 8.2 %	
350 r	Series No. of animals Hypophysis Prostate Seminal vesicles	AII a 8 + 4.6± 3.7 % *−41.2±10.5 %* *−62.8± 9.7 %*	AIII b 8 + 4.9± 2.7 % *−36.0± 5.8 %* *−53.2± 6.2 %*	AIV a 8 + 7.6± 4.5 % *−23.0± 6.4 %* *−42.5± 9.8 %*	AV b 8 + 6.3± 3.7 % −22.2± 8.3 % *−48.6±14.7 %*	AVI b 15 + 9.0± 3.3 % *−18.5± 5.9 %* *−33.0±10.1 %*	AVII c 8 *+12.4± 2.6 %* *−32.0± 4.4 %* *−43.9± 9.8 %*	AVIII (fig. 16) 30 + 6.5± 2.5 % *−22.6± 3.4 %* *−37.8± 7.9 %*	AIX b (fig. 18) 31 *+19.3± 2.6 %* + 1.4± 5.3 % − 2.8±10.1 %	AX c 8 *+16.1± 5.2 %* *−11.0± 3.6 %* −32.4±13.5 %		
	Series No. of animals Hypophysis Prostate Seminal vesicles							BVIII 17 *+10.4± 2.6 %* *−25.0± 4.7 %* *−44.3± 9.0 %*	BIX b 18 *+21.1± 2.9 %* − 1.8± 5.5 % −14.6±14.0 %			
500 r	Series No. of animals Hypophysis Prostate Seminal vesicles	AII b 6 + 0.7±10.5 % *−61.4± 8.8 %* *−84.3± 6.2 %*	AIII c 8 +10.9± 4.7 % *−30.0± 8.4 %* *−58.9±14.2 %*	AIV b 8 *+ 9.4± 2.6 %* *−32.9± 6.0 %* *−59.0± 9.2 %*		AVI c 13 *+10.7± 2.8 %* −10.5± 6.7 % −26.1±10.1 %	AVII d 6 +10.4± 3.5 % *−25.4± 8.4 %* −36.5±12.7 %			AX d 9 *+20.4± 5.5 %* − 2.4± 3.7 % −20.6±15.0 %		
700 r	Series No. of animals Hypophysis Prostate Seminal vesicles	AII c 7 + 8.1± 6.9 % *−60.8± 7.9 %* *−85.5± 5.5 %*		AIV c 8 *+11.5± 3.5 %* *−40.5± 6.2 %* *−65.3± 8.5 %*								

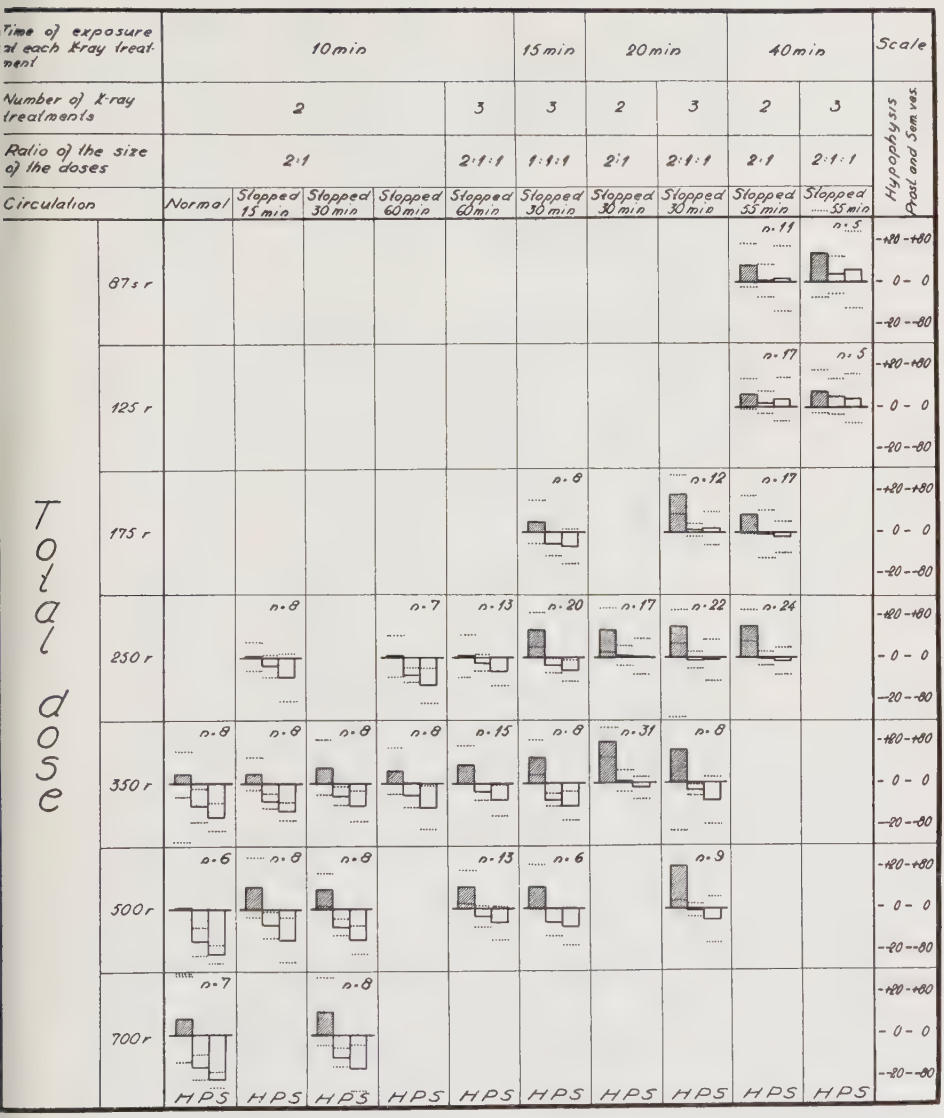


Fig. 13. Chart of difference between weight of hypophysis (H), prostate (P) and seminal vesicles (S) in rats with X-ray treated testes and in normal rats. The heights of the columns give the difference in per cent of normal weight. Upward columns show increase, downward columns decrease. The dotted lines correspond to 3 times the standard error of the difference. n = number of X-ray treated animals.

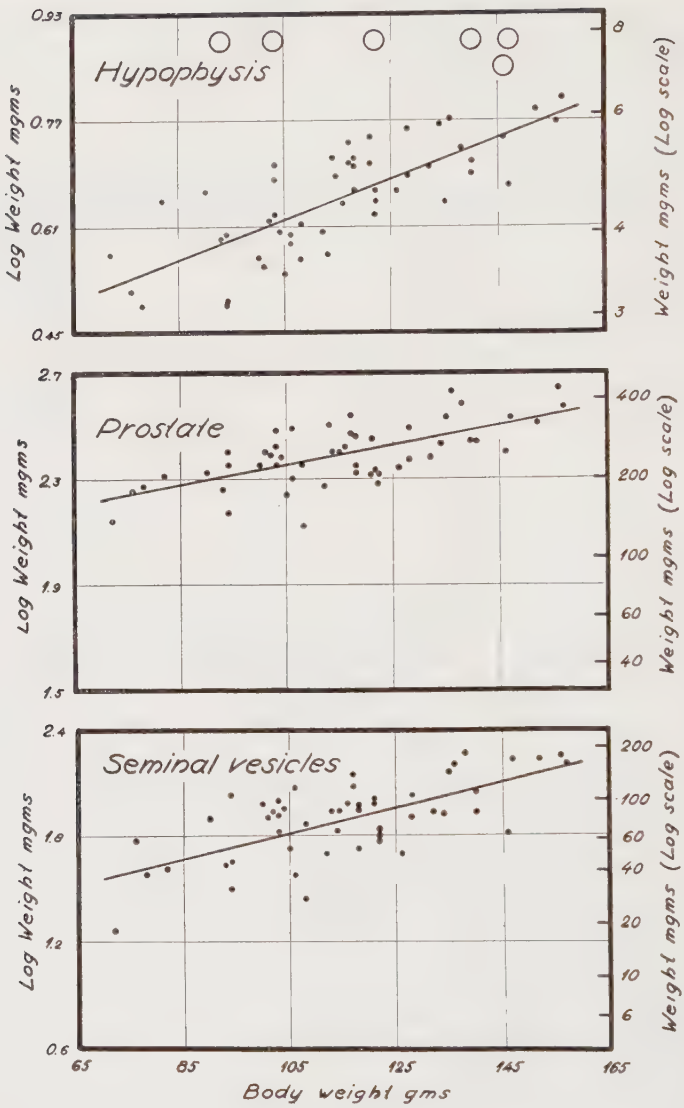


Fig. 14. Weight of hypophysis, prostate and seminal versicles of normal rats according to body weight. Series AI. • Observed weight. ——— Calculated weight. Formulas (16), (17) and (18). The circles, ○, in the hypophysis-chart refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

Normal animals, Series BI.

Hypophysis. $y = +0.396 + 0.00246 . x$ (20)
$a_1 \pm \varepsilon (a_1) = +0.00246 \pm 0.00053$

Prostate. $y = +1.810 + 0.00419 . x$ (21)
$a_1 \pm \varepsilon (a_1) = +0.00419 \pm 0.00143$

Seminal vesicles. $y = +0.864 + 0.00775 . x$ (22)
$a_1 \pm \varepsilon (a_1) = +0.00775 \pm 0.00287$

Series AVIII.

Hypophysis. $y = +0.330 + 0.00303 . x$ (23)
$a_1 \pm \varepsilon (a_1) = +0.00303 \pm 0.00041$

Prostate. $y = +1.692 + 0.00507 . x$ (24)
$a_1 \pm \varepsilon (a_1) = +0.00507 \pm 0.00085$

Seminal vesicles. $y = +0.463 + 0.0106 . x$ (25)
$a_1 \pm \varepsilon (a_1) = +0.0106 \pm 0.0021$

Testicles. $y = +2.252 + 0.00313 . x$ (26)
$a_1 \pm \varepsilon (a_1) = +0.00313 \pm 0.00088$

Series AIXb.

Hypophysis. $y = +0.392 + 0.00292 . x$ (27)
$a_1 \pm \varepsilon (a_1) = +0.00292 \pm 0.00036$

Prostate. $y = +1.947 + 0.00389 . x$ (28)
$a_1 \pm \varepsilon (a_1) = +0.00389 \pm 0.00116$

Seminal vesicles. $y = +1.041 + 0.00728 . x$ (29)
$a_1 \pm \varepsilon (a_1) = +0.00728 \pm 0.00224$

Testicles. $y = +2.416 + 0.00215 . x$ (30)
$a_1 \pm \varepsilon (a_1) = +0.00215 \pm 0.00092$

As with the investigation into experimental cryptorchism, it was desirable always to reckon with the same values for a_1 when dealing with the same organ. The values for a_1 appearing in the regression equations for the normal series, AI, have been compared with a_1 in the corresponding equations for other series. The comparison has been confined to those series where the number of test animals was greatest, Series AVIII and AIXb, as well as the normal series BI. The difference was as a rule negligible, and never exceeded 1.5 times the standard error of the difference, for which reason the simplification mentioned was deemed permissible.

Regression equations have been calculated where a_1 was equal to a_1 in corresponding equations for the normal series AI (16), (17), (18) and (19). The equations given below are only those for the regression lines appearing in figs. 16—21, 23 and 24.

Series AVIII (figs. 16 and 23).

Hypophysis.	$y = +0.328 + 0.00306 . x$	(31)
Prostate.	$y = +1.842 + 0.00378 . x$	(32)
Seminal vesicles.	$y = +0.851 + 0.00724 . x$	(33)
Testicles.	$y = +2.444 + 0.00148 . x$	(34)

Series AIXa (fig. 17).

Hypophysis.	$y = +0.355 + 0.00306 . x$	(35)
Prostate.	$y = +1.966 + 0.00378 . x$	(36)
Seminal vesicles.	$y = +1.067 + 0.00724 . x$	(37)

Series AIXb (figs. 18 and 24).

Hypophysis.	$y = +0.377 + 0.00306 . x$	(38)
Prostate.	$y = +1.959 + 0.00378 . x$	(39)
Seminal vesicles.	$y = +1.045 + 0.00724 . x$	(40)
Testicles.	$y = +2.481 + 0.00148 . x$	(41)

Series AXa (fig. 19).

Hypophysis.	$y = +0.374 + 0.00306 . x$	(42)
Prostate.	$y = +1.975 + 0.00378 . x$	(43)
Seminal vesicles.	$y = +1.083 + 0.00724 . x$	(44)

Series AXb (fig. 20).

Hypophysis.	$y = +0.363 + 0.00306 . x$	(45)
Prostate.	$y = +1.939 + 0.00378 . x$	(46)
Seminal vesicles.	$y = +1.044 + 0.00724 . x$	(47)

Series AXId (fig. 21).

Hypophysis.	$y = +0.363 + 0.00306 . x$	(48)
Prostate.	$y = +1.951 + 0.00378 . x$	(49)
Seminal vesicles.	$y = +1.039 + 0.00724 . x$	(50)

The regression equations for normal animals, Series BI.

Hypophysis.	$y = +0.319 + 0.00306 . x$	(51)
Prostate.	$y = +1.862 + 0.00378 . x$	(52)
Seminal vesicles.	$y = +0.929 + 0.00724 . x$	(53)

The animals in the series AIIa—AIIc had received X-ray treatment twice. The time of exposure each time was 10 minutes. The ratio of the doses on the two occasions was 2: 1. The total doses were 350 r, and 500 and 700 r respectively. As can be seen from table 1, after p. 32, and fig. 13, this treatment sets up an appreciable inhibition of the growth of the prostate and the seminal vesicles. The hypophyses

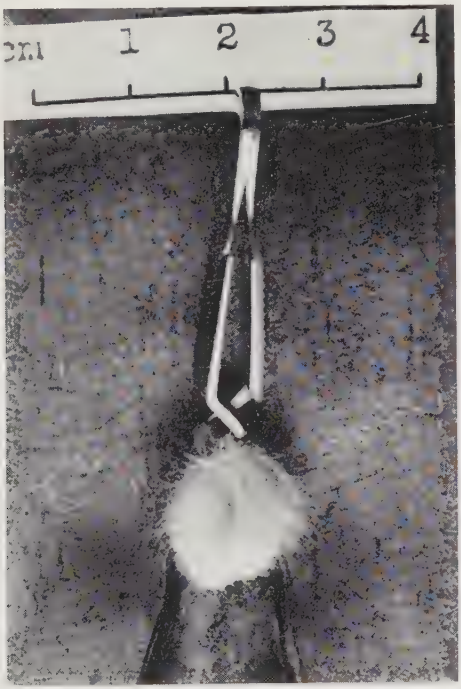


Fig. 15.

display no sure weight increase. The aim of the treatment was, as has been said, to try and induce weight increase in the hypophysis without at the same time inhibiting the growth of prostate and seminal vesicles. This does not seem to be possible with this form of X-ray treatment.

Investigations have been made into the effect of stopping the blood circulation in the testicles during the X-ray treatment, and also the effect of fractionating and protraction of the dose.

The circulation was stopped by placing an artery clip on the neck of the scrotum (fig. 15) after the testicles had been brought down. In the series AIIIa—AIIIc (table 1, after p. 32, and fig. 13), the circulation was stopped for 15 minutes. Time of exposure 10 mins. The circulation was stopped during the treatment and during the 5

minutes immediately preceding. In the series AIVa—AIVc, and AVa—AVc, it was stopped for 30 and 60 minutes respectively. This period covered the time of treatment, the 5 minutes immediately before, and the 15 and 45 minutes respectively immediately following. Exposure time in all cases 10 mins. As can be seen from table 1, after p. 32, and fig. 13, a stopping of the circulation seems to influence the effect. The tendency to weight increase in the hypophysis is more marked. When the circulation was stopped for 30 minutes, a total dose of 500 r produced a statistically significant weight increase in the hypophysis. At the same time, the growth inhibition of prostate and seminal vesicles seems to diminish.

A further fractionating of the dose, 3 treatments instead of 2, does not seem to produce any change. Series AVIa—AVIc. The ratio of the X-ray doses was 2 : 1 : 1. The circulation was stopped.

The effect of protraction, on the other hand, seems to be pronounced. An increase of the exposure time from 10 to 15 minutes set up as early as at a total dose of 250 r a statistically significant weight increase in the hypophysis. Series AVIIa –AVIId. Number of treatments 3. Ratio of X-ray doses 1 : 1 : 1. Circulation stopped.

With a further protraction, it was possible to produce a statistically significant weight increase in the hypophysis without inhibiting the growth of prostate and seminal vesicles. Series AIXa, AIXb (figs. 17 and 18). Histological examination revealed no marked castration changes in the hypophyses. In only two of the 9 hypophyses examined were a few isolated signet-ring cells observed (fig. 18). The weight of the testicles was considerably lowered (figs. 22 and 24), the germinal tissue atrophied to a high degree — the interstitial cells, on the other hand, well preserved (figs. 52—55). Time of exposure 20 mins. The ratio of the X-ray doses 2: 1. Circulation stopped.

Series BIXb were treated in the same way as Series AIXb. The results tallied.

The effect of stopping the circulation was also investigated with an exposure time of 20 mins. Series AVIII, AIXb, BVIII and BIXb (figs. 16 and 18). More animals were used than in the series AII—AV, to try and make the effect statistically significant; this is shown in figs. 25 and 26, and table 2, p. 49. The stopping of the circulation during treatment stimulates the weight increase in the hypophysis and diminishes the weight inhibition in prostate and seminal vesicles. The differences are statistically significant. No certain change sets in as regards the weight of the testicles (figs. 23 and 24).

Here, too, when the exposure time is 20 mins, further fractionating of the dose — 3 treatments instead of 2 — does not seem to alter the effect (figs. 19 and 20).

The exposure time was raised to 40 mins. The number of treatments was 2 in the series AXIa—AXId (fig. 21), and 3 in the series AXIIa

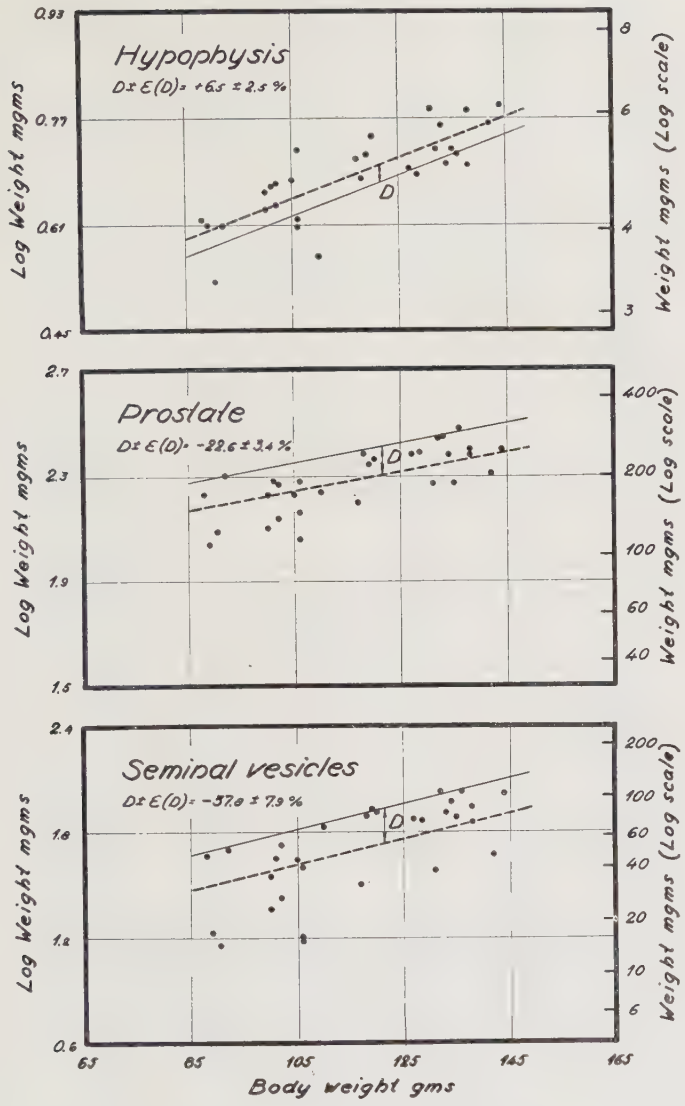


Fig. 16. Weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Series AVIII. Dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation intact. • Observed weight. — — — — Calculated weight. Formulas (31), (32) and (33). ——— Calculated normal weight. Formulas (16), (17) and (18).

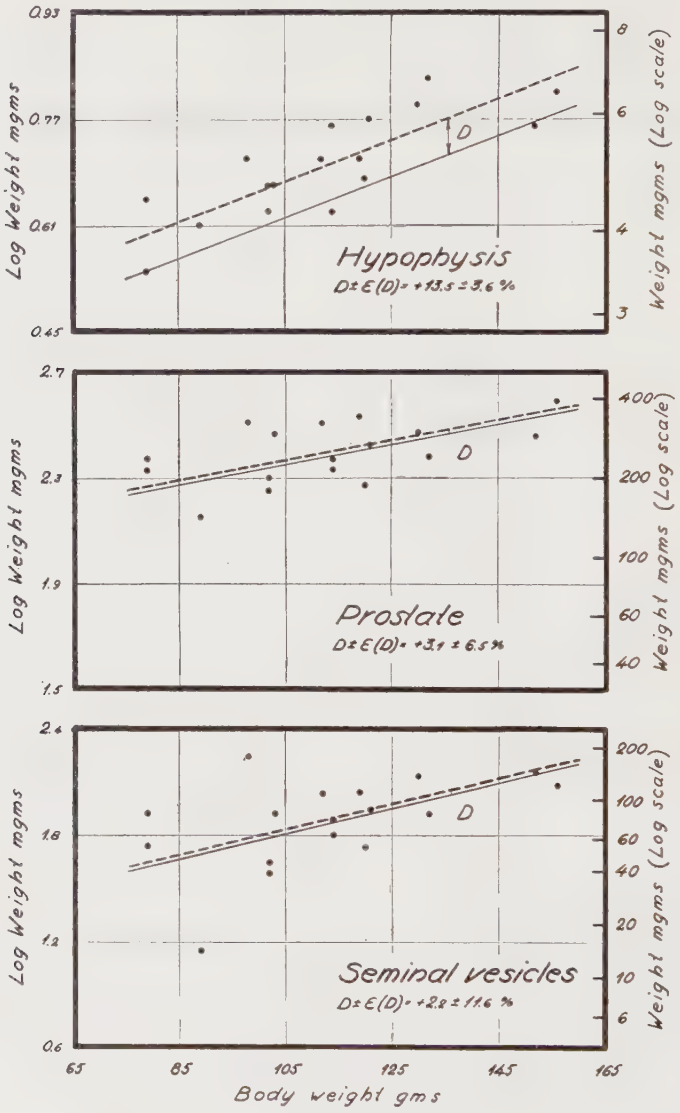


Fig. 17. Weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Series AIX a. Dose 167 + 83 = 250 r. Time of exposure 20 min. Circulation stopped. • Observed weight. — — — — Calculated weight. Formulas (35), (36) and (37). ——— Calculated normal weight. Formulas (16), (17) and (18).

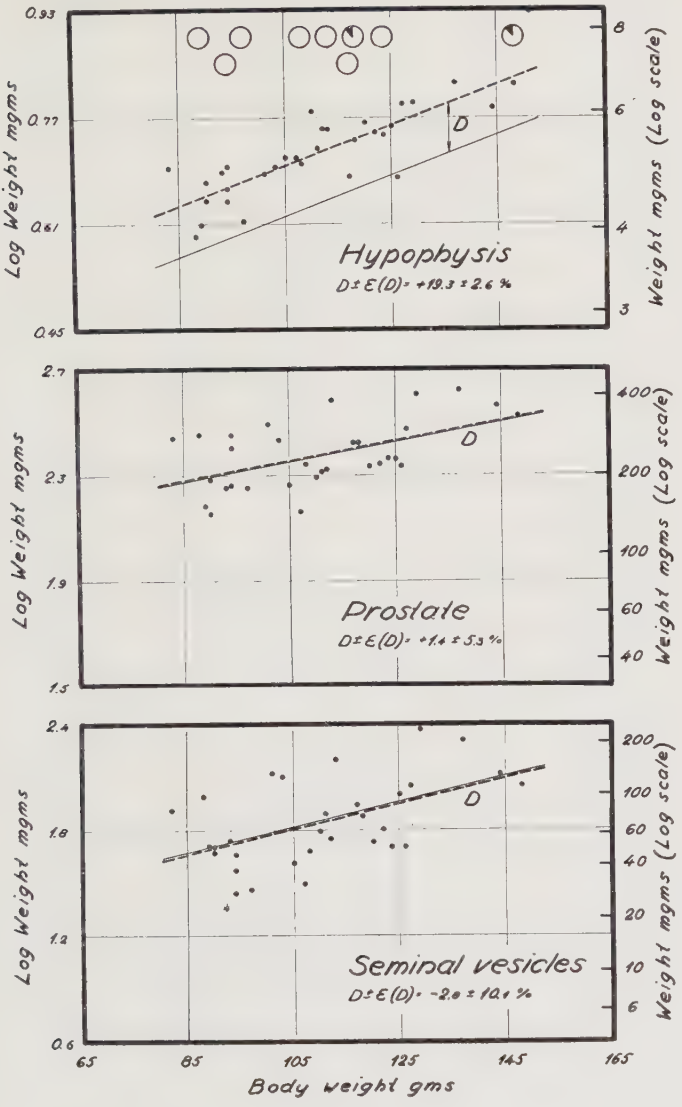


Fig. 18. Weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Series AIX b. Dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. • Observed weight. — — — — Calculated weight. Formulas (38), (39) and (40). ——— Calculated normal weight. Formulas (16), (17) and (18). The circles, ○, in the hypophysis chart refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

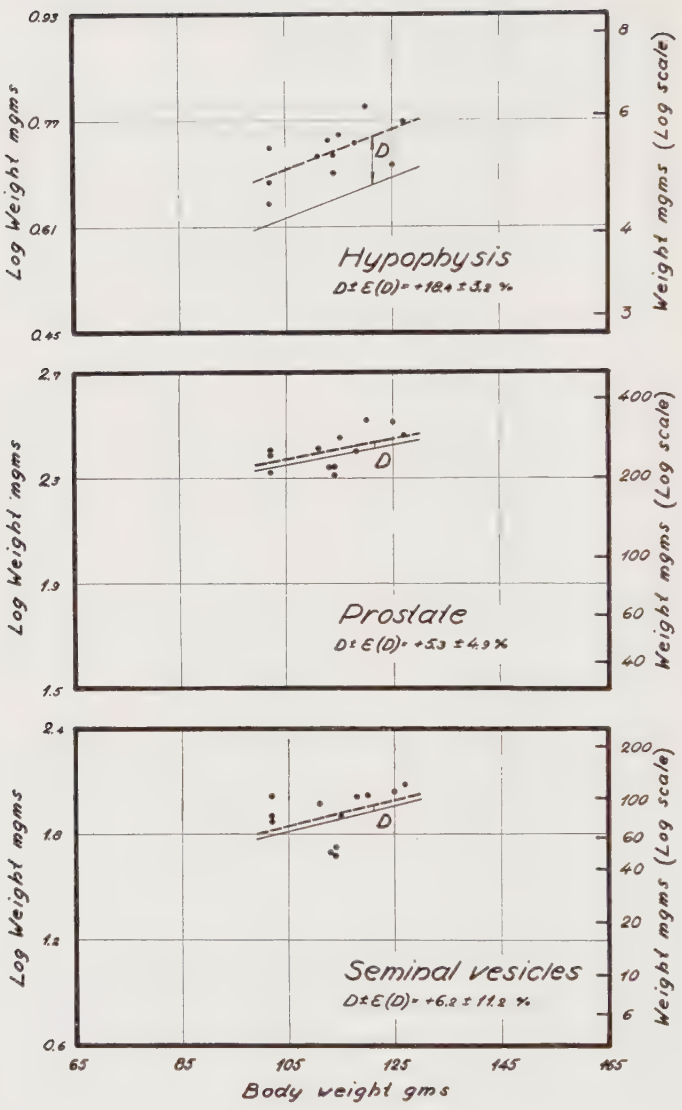


Fig. 19. Weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Series A X a. Dose 87 + 44 + 44 = 175 r. Time of exposure 20 min. Circulation stopped. • Observed weight. — — — — Calculated weight. Formulas (42), (43) and (44). ——— Calculated normal weight. Formulas (16), (17) and (18).

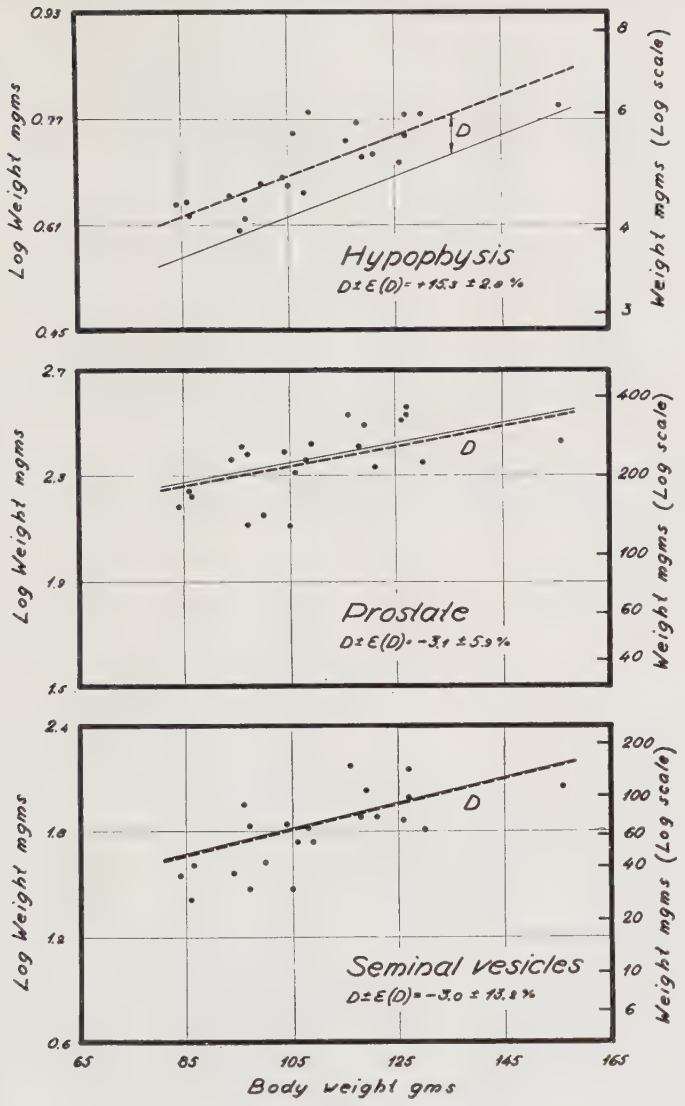


Fig. 20. Weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Series A X b. Dose 125 + 63 + 62 = 250 r. Time of exposure 20 min. Circulation stopped. • Observed weight. — — — — Calculated weight. Formulas (45), (46) and (47). ——— Calculated normal weight. Formulas (16), (17) and (18).

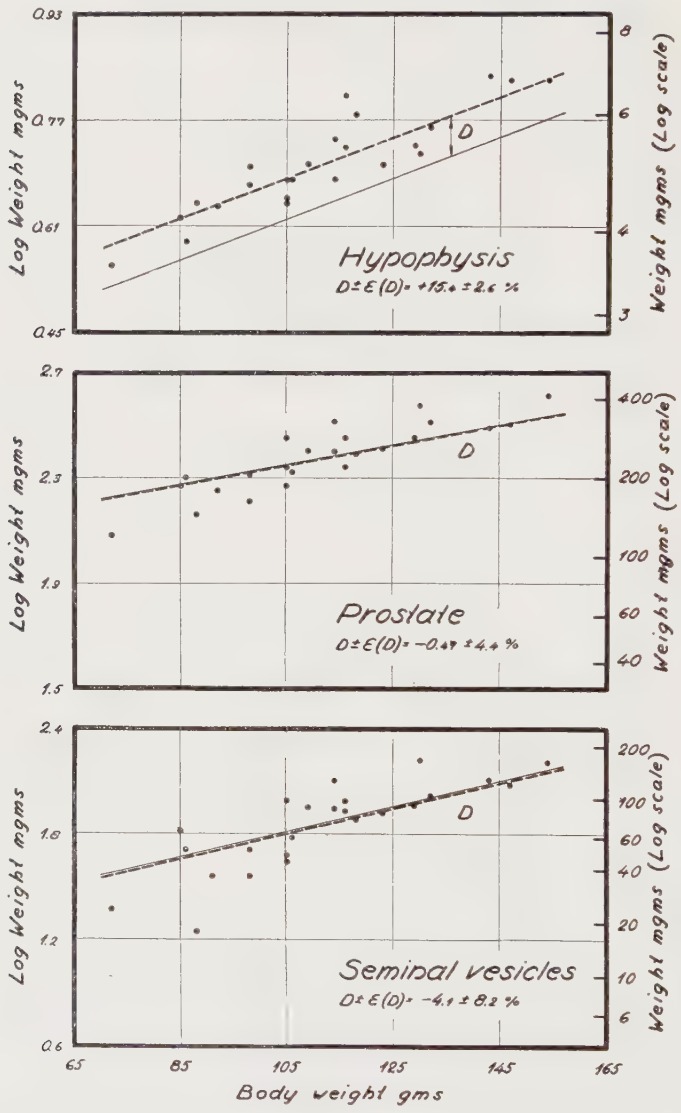


Fig. 21. Weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Series A XI d. Dose 167 + 83 = 250 r. Time of exposure 40 min. Circulation stopped. • Observed weight. — — — — Calculated weight. Formulas (48), (49) and (50). ——— Calculated normal weight. Formulas (16), (17) and (18).

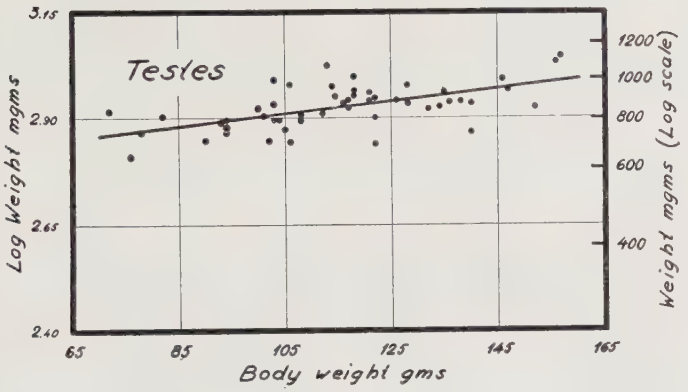


Fig. 22. Weight of testes of normal rats according to body weight. Series A I. • Observed weight. ——— Calculated weight. Formula (19).

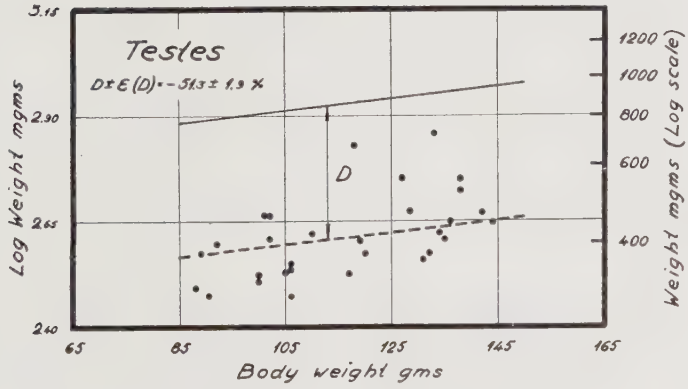


Fig. 23. Weight of testes of X-rayed rats according to body weight. Series A VIII. Dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation intact. • Observed weight. — — — — Calculated weight. Formula (34). ——— Calculated normal weight. Formula (19).

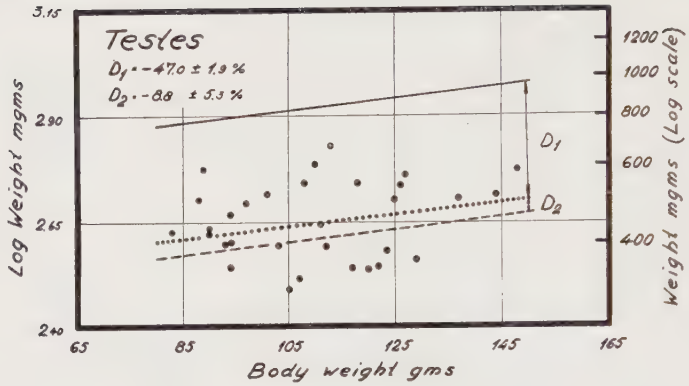


Fig. 24. Weight of testes of X-rayed rats according to body weight. Series A IX b. Dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. • Observed weight. ····· Calculated weight. Formula (41). ——— Calculated normal weight. Formula (19). — — — Calculated weight, X-rayed, circulation intact. Formula (34).

and AXIIb. With the smaller doses — total dose 87.5 and 125 r — there was not only an increase of the hypophysis weight but also of the prostate compared with the weight of these organs in normal animals. As table 3, p. 50, shows, the weight increase of the hypophysis is statistically significant. The probability of significance for the prostate is 0.99 and for the seminal vesicles 0.90.

The effect of protracting and fractionating the X-ray dose has been given in tables 4 and 5, p. 51 and 52. They show that protraction has the same effect as stopping the blood circulation through the testicles during treatment: namely a weight increase in the hypophysis and diminished inhibition of the growth of prostate and seminal vesicles. The differences are statistically significant. No significant difference was observed as regards fractionating.

Discussion. By appropriate X-ray treatment of the testicles of young male rats, it is possible to induce atrophy of the germinal tissue without inhibiting the growth of prostate and seminal vesicles. Here, as in castration, a weight increase occurs in the hypophysis. The interstitial cells in such testicles are well preserved. Thus, a hormone is formed in the germinal tissue which, under normal circumstances, prevents this weight increase in the hypophysis, and another (testosterone) in the interstitial cells, which stimulates the growth of the accessory sex glands.

Stopping the circulation through the testicles during X-ray treatment, and protracting the dose, increases the difference in sensitivity to irradiation between the germinal and interstitial tissues.

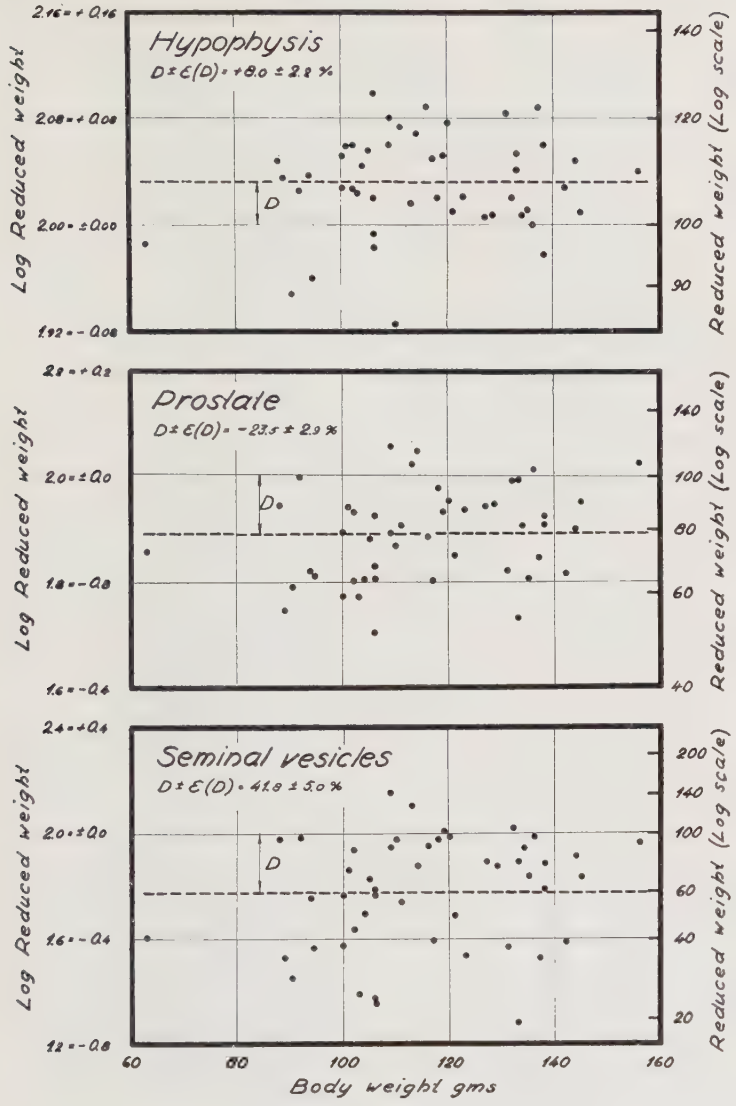


Fig. 25. Reduced weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Normal weight = 100. Series A VIII + B VIII. Dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation intact. • Observed weight. — — — — Calculated weight.

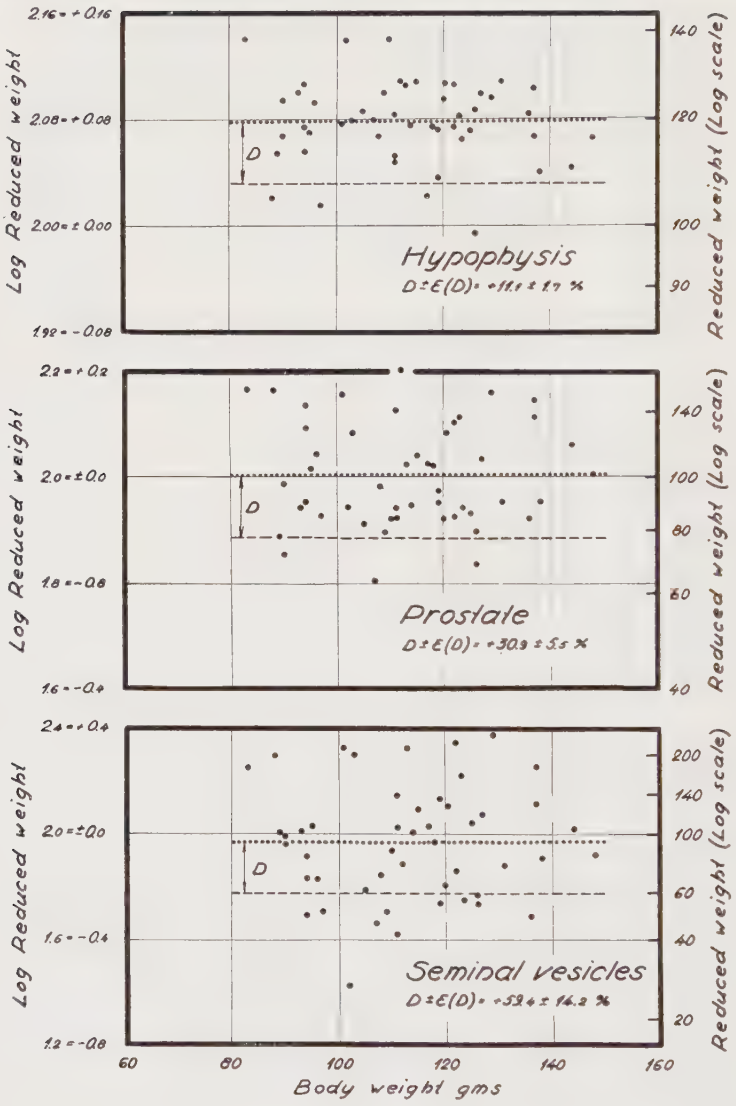


Fig. 26. Reduced weight of hypophysis, prostate and seminal vesicles of X-rayed rats according to body weight. Normal weight = 100. Series A IX b + B IX b. Dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. • Observed weight. ····· Calculated weight. ——— Calculated weight, X-rayed, circulation intact.

Table 2. *The effect of stopping the circulation of the blood through the testicles during X-ray treatment. Difference (D) between the weight of hypophysis, prostate and seminal vesicles in rats with circulation, stopped during X-ray treatment, and in rats treated with circulation normal. The difference is expressed in % of the weight in the latter. The sign + shows increase, the sign — decrease. The difference is given in italics if it is equal to or greater than 3 times the standard error of the difference.*

Circulation		Total dose	No. of X-ray treatments	Ratio of size of doses	Time of exposure in each X-ray treatment	Hypophysis $D \pm \varepsilon(D)$	Prostate $D \pm \varepsilon(D)$	Seminal vesicles $D \pm \varepsilon(D)$
Stopped 30 mins.	Normal							
Ser. AIX b (31 animals)	Ser. AVIII (30 animals)	350 r.	2	2 : 1	20 min.	*+11.9±2.4 %*	*+30.9±7.3 %*	*+56.4±18.8 %*
Ser. BIX b (18 animals)	Ser. BVIII (17 »)	»	»	»	»	*+ 9.7±2.3 %*	*+30.9±6.8 %*	+64.0±27.7 %
					Mean difference	*+11.1±0.6 %*	*+30.9±2.7 %*	*+59.2± 4.1 %*

Table 3. *Difference (D) between the weight of hypophysis, prostate and seminal vesicles in animals with testicles treated with X-ray, and in normal animals (Series AI, 51 animals). The difference is expressed in % of the normal weight. The sign + shows increase. The difference is given in italics if it is equal to or greater than three times the standard error of the difference. p = probability of the differences' significance.*

Series	Total dose	No. of X-ray treatments	Ratio of size of the doses	Time of exposure in each X-ray treatment	Circulation	Hypophysis $D \pm \varepsilon_D$	Prostate $D \pm \varepsilon_D$	Seminal vesicles $D \pm \varepsilon_D$
AXI a (11 animals)	87.5 r	2	2 : 1	40 mins.	Stopped 55 mins.	+ 8.2 ± 3.6 %	− 3.2 ± 10.9 %	8.6 ± 21.6 %
AXI b (17 animals)	125 r	2	2 : 1	»	»	+ 6.5 ± 2.6 %	− 9.3 ± 7.0 %	15.3 ± 14.2 %
AXII a (5 animals)	87.5 r	3	2 : 1 : 1	»	»	+ 14.8 ± 7.0 %	− 17.4 ± 12.6 %	24.8 ± 25.1 %
AXII b (5 animals)	125 r	3	2 : 1 : 1	»	»	+ 8.0 ± 3.5 %	− 22.0 ± 11.0 %	16.1 ± 15.7 %
					Mean difference	+ *9.20* ± *2.0* %	+ 12.6 ± 4.9 % $p = 0.99$	+ 16.0 ± 9.5 % $p = 0.90$

Table 4. *Effect of protraction. Difference (D) between weight of hypophysis, prostate and seminal vesicles in rats receiving X-ray treatment with an exposure period of 20 mins., and in rats receiving X-ray treatment with an exposure period of 10 mins. The difference is expressed in % of weight in the latter. The sign + shows increase, the sign — decrease. The difference is given in italics if it is equal to or greater than 3 times the standard error of the difference.*

Time of exposure in each X-ray treatment		Total dose	No. of X-ray treatments	Ratio of size of the doses	Circulation	Hypophysis $D \pm \varepsilon(D)$	Prostate $D \pm \varepsilon(D)$	Seminal vesicles $D \pm \varepsilon(D)$
20 mins.	10 mins.							
Ser. AVIII (30 animals)	Ser. AII a (8 animals)	350 r	2	2 : 1	[1]	+ 1.8 ± 3.6 %	+ 31.7 ± 23.5 %	- 66.9 ± 44.7 %
Ser. AIX b (31 animals)	Ser. AIV a (8 animals)	350 r	2	2 : 1	[2]	+ 10.8 ± 4.5 %	+ 31.7 ± 11.8 %	+ 69.2 ± 31.2 %
Ser. AX b (22 animals)	Ser. AVI a (13 animals)	250 r	3	2 : 1 : 1	[2]	*+ 13.6 ± 3.6 %*	+ 5.5 ± 7.5 %	+ 32.6 ± 22.2 %
Ser. AX c (8 animals)	Ser. AVI b (15 animals)	350 r	3	2 : 1 : 1	[2]	*+ 26.6 ± 6.1 %*	+ 9.3 ± 7.9 %	+ 0.9 ± 21.5 %
Ser. AX d (9 animals)	Ser. AVI c (13 animals)	500 r	3	2 : 1 : 1	[2]	+ 8.8 ± 5.0 %	+ 9.1 ± 8.0 %	+ 7.5 ± 23.7 %
					Mean difference	*+ 11.3 ± 1.4 %*	*+ 19.4 ± 6.3 %*	*+ 41.8 ± 13.3 %*

[1] Normal. [2] Stopped 30 mins.

Table 5. *Effect of fractionating. Difference (D) between weight of hypophysis, prostate and seminal vesicles in rats receiving X-ray treatment 3 times, and those receiving it twice. The difference is expressed in % of weight in the latter. The sign + shows increase, the sign — decrease. The difference is never equal to or greater than 2.5 times the standard error of the difference.*

Number of X-ray treatments and ratio of the doses		Total dose	Time of exposure in each X-ray treatment	Circulation	Hypophysis D ± ε (D)	Prostate D ± ε (D)	Seminal vesicles D ± ε (D)
3 (2 : 1 : 1)	2 (2 : 1)						
Ser. AVI b (15 animals)	Ser. AIV a (8 animals)	350 r	10 mins.	[1]	+ 1.3 ± 4.6 %	+ 5.8 ± 10.8 %	+ 16.6 ± 25.0 %
Ser. AVI c (13 animals)	Ser. AIV b (8 animals)	500 r	10 mins.	[1]	+ 1.2 ± 2.5 %	+ 33.4 ± 14.5 %	+ 80.0 ± 45.5 %
Ser. AX b (22 animals)	Ser. AIX a (17 animals)	250 r	20 mins.	[1]	+ 1.6 ± 3.2 %	— 0.14 ± 7.8 %	— 0.9 ± 15.9 %
Ser. AX c (8 animals)	Ser. AIX b (31 animals)	350 r	20 mins.	[1]	— 3.2 ± 4.2 %	— 10.0 ± 4.8 %	— 34.3 ± 13.9 %
Ser. AXII a (5 animals	Ser. AXI a (11 animals)	87.5 r	40 mins.	[2]	+ 6.0 ± 6.9 %	+ 13.8 ± 16.5 %	+ 15.0 ± 31.4 %
Ser. AXII b (5 animals)	Ser. AXI b (17 animals)	125 r	40 mins.	[2]	+ 1.4 ± 3.3 %	+ 11.6 ± 11.5 %	+ 0.7 ± 16.8 %
			Mean difference		+ 0.58 ± 1.7 %	+ 5.7 ± 3.8 %	+ 5.9 ± 8.9 %

[1] Stopped 30 mins. [2] Stopped 55 mins.

That another hormone is formed in the testicles, over and above the one stimulating the growth of prostate and seminal vesicles, is confirmed by the observations made in the changes in irradiation technique which have been mentioned. In both cases the weight increase of the hypophysis is raised, and at the same time the inhibition of the growth of prostate and seminal vesicles, otherwise set up by X-ray treatment, is lessened. The weight increase of the hypophysis thus can not have been caused by a lowered production of the testicle hormone stimulating the growth of the accessories. The lessened inhibition in prostate and seminal vesicles is probably due to an increase of the gonadotropic function of the hypophysis. Castration causes an increased formation of gonadotropic hormon in this organ. When administered to non-hypophysectomized males, it stimulates the testicles to increased formation of the testicle hormone regulating the growth of the accessories; it does this whether it is of the follicle-stimulating or the luteinizing type. Since the cessation of the germinal tissue's internal secretion sets up, as castration does, an enlargement of the hypophysis, there is reason to suppose that it also sets up an increased production of gonadotropic hormone. The heightened action of gonadotropic hormone would, if this is so, release a greater internal secretion in the interstitial cells, and more growth of the prostate and seminal vesicles. As regards protraction, other factors, too, may be of importance. Protraction means a lessening of the rays' intensity, which may possibly lessen the injurious effect of the treatment on the interstitial cells.

We are supported in saying the cessation of the germinal tissue's internal secretion via the hypophysis releases an enhanced production of testosterone by the fact that, when the time of exposure for each X-ray treatment is increased to 40 mins, a total dose of 87.5 and 125 r sets up not only increase of the hypophysis weight but also of that of the prostate compared with the weight of these organs in normal animals.

For a definitive proof that the cessation of the internal secretion of the germinal tissue via the hypophysis sets up a greater formation of the testicle hormone stimulating the growth of the accessories, it seems necessary to compensate the cessation by administering the hypothetical hormone and seeing if the weight increase in the prostate and seminal vesicles sets in or not.

Summary. A study has been made of changes in hypophysis and accessories, after injury to the germinal tissue of the testicles. By treating the testicles of young male rats in a certain way with X-rays, it has been possible to induce atrophy of the germinal tissue without inhibiting the growth of prostate and seminal vesicles. Here, as at castration, there is weight increase of the hypophysis. The interstitial cells in such testicles are well preserved. From this is concluded that

a hormone is formed in the germinal tissue which under normal conditions prevents this increase in the hypophysis. A hormone (testosterone) is formed in the interstitial cells which stimulates the growth of the accessory sex glands.

By stopping the blood circulation through the testicles during X-ray treatment, and by protraction of the dose, it is possible to enhance the difference in X-ray sensitivity between the germinal and the interstitial tissues. The changes mentioned as to irradiation technique raise the weight increase in the hypophysis, and lessen the growth inhibition of prostate and seminal vesicles which otherwise sets in after X-ray treatment. When the time of exposure in each treatment was increased to 40 mins, and the total dose was 87.5 and 125 r, there was not only weight increase in the hypophysis but also in the prostate, compared with the weight of these organs in normal animals. The causes of the lessened inhibition and of the weight increase in the accessory sex glands are discussed, and asserted to be due to cessation of the germinal tissue's internal secretion — via an increased formation of gonadotropic hormone in the hypophysis — releasing a greater production of the testicle hormone stimulating the accessory sex glands.

Chapter 5. The Hormone of the Germinal Tissue.

Introduction. It has been shown in the previous chapter that X-ray treatment of the testicles performed in a certain way is able to induce atrophy of the germinal tissue without inhibiting the growth of the prostate and seminal vesicles. In so doing, weight increase is set up in the hypophysis. The interstitial cells are well preserved. It has also been shown that administering testosterone to young castrate male rats in such quantity as to raise the weight of the prostate and seminal vesicles somewhat over the weight of these organs in normal animals does not prevent the increase in weight which appears in the hypophysis after castration. Thus the hormone which is formed in the germinal tissue is characterized, in relation to the hormone (testosterone) formed in the interstitial cells, by a stronger effect on the weight increase of the hypophysis which sets in after castration, compared with that on the accessory sex glands.

Although the hypophysis in both the groups of animals mentioned are, as in castrates, enlarged, they display no marked histological castration changes. The administration of increasing doses of the germinal tissue hormone to castrate male rats ought therefore in the first place to effect the weight increase of the hypophysis. The appearance of so-called castration cells is probably effected secondarily, or not at all.

Testicle extracts have been prepared and tested out on castrate male rats, in order to isolate the hormone formed in the germinal tissue.

In so doing, the effect of these extracts on hypophysis and on prostate and seminal vesicles has been compared with the corresponding effect of synthetic testosterone and testosterone propionate.

Methods in testing the biological activity. Young male white rats were castrated at a weight of about 30 gm. Every day they were given a subcutaneous injection of 0.10 cc of the solution to be tested; they received the same dose during the whole time of the test. Four animals were used for each dose. The doses were in the ratio of 1: 2: 4: 8: 16: 32 and so on. After 3 weeks the animals were killed and examined. The prostate, seminal vesicles and hypophysis were prepared and weighed. The hypophysis weight was calculated per 100 gm body weight. These organs were also examined histologically. As regards the histological technique, see p. 18. In this examination, all that was taken into account was the presence of so-called castration cells.

The substances or extracts tested were dissolved in sesame oil. The solution was prepared by first dissolving the substance in peroxide-free ethyl ether. Sesame oil was added. The ether was evaporated off by heating to 100° C in a vacuum.

The effect of testosterone and testosterone propionate is shown in figs. 27 and 28. The administration of either causes the weight increase set up by castration to disappear, as also the so-called castration cells (figs. 56—58). The castration atrophy of prostate and seminal vesicles is affected. The diagram shows that the doses affecting the castration changes in the hypophysis also affect the weight of the prostate and seminal vesicles.

Preparation and effect of testis extracts. The postulated hormone of the germinal tissue has, like the known sex hormones, been taken to be of a lipoid nature. Methods of extraction and purifying usable for all known sex hormones were chosen.

Starting from the analogy between the hormones of the ovaries and the testicles, such methods were used in the attempts to separate testosterone and the hormone of the germinal tissue as make possible a chemical separation of progesterone and estrogen.

Extracts of bull testes. Bull testes were used as basis. They were frozen after the animal had been slaughtered. The coverings and epididymis were cut off. The testes were ground.

The following process was used in preparing testis extract (scheme A, p. 58):

1) 50 kg ground testis substance were extracted with 100 litres of 95 % alcohol. This was filtered and the solid parts thrown away.

2) The clear filtrate was concentrated in a vacuum to about 5 litres. 500 ccs of this was taken and evaporated to dryness in a Gaede and Straub's distillation apparatus (1925). Fraction BI. 22.1 gm fraction

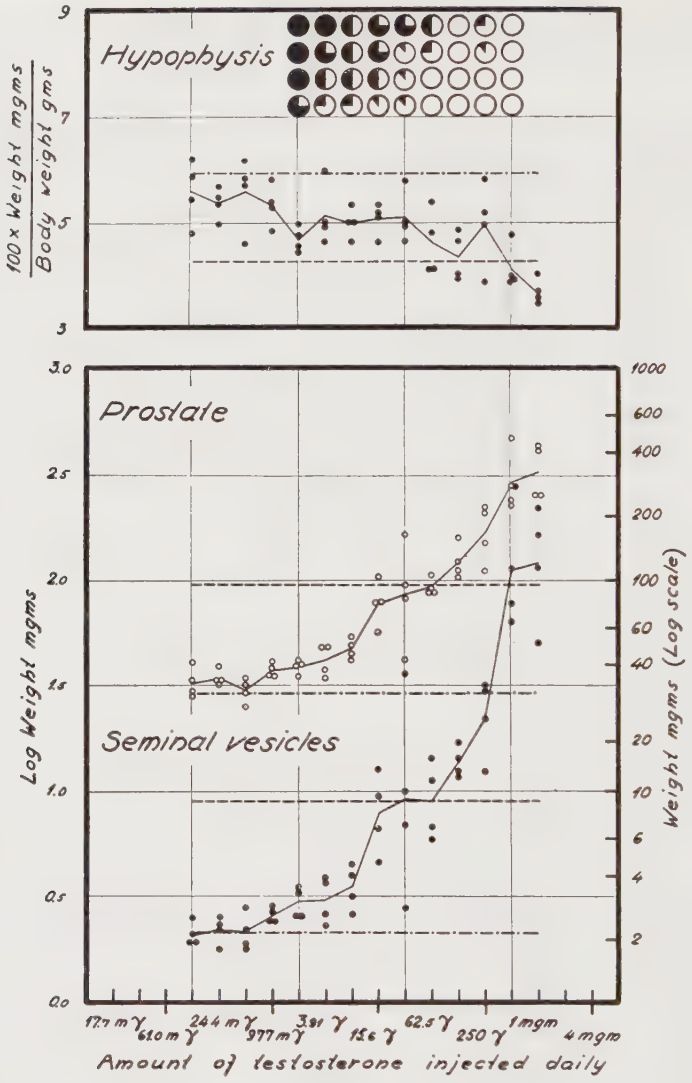


Fig. 27. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with testosterone for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, in the hypophysis chart refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

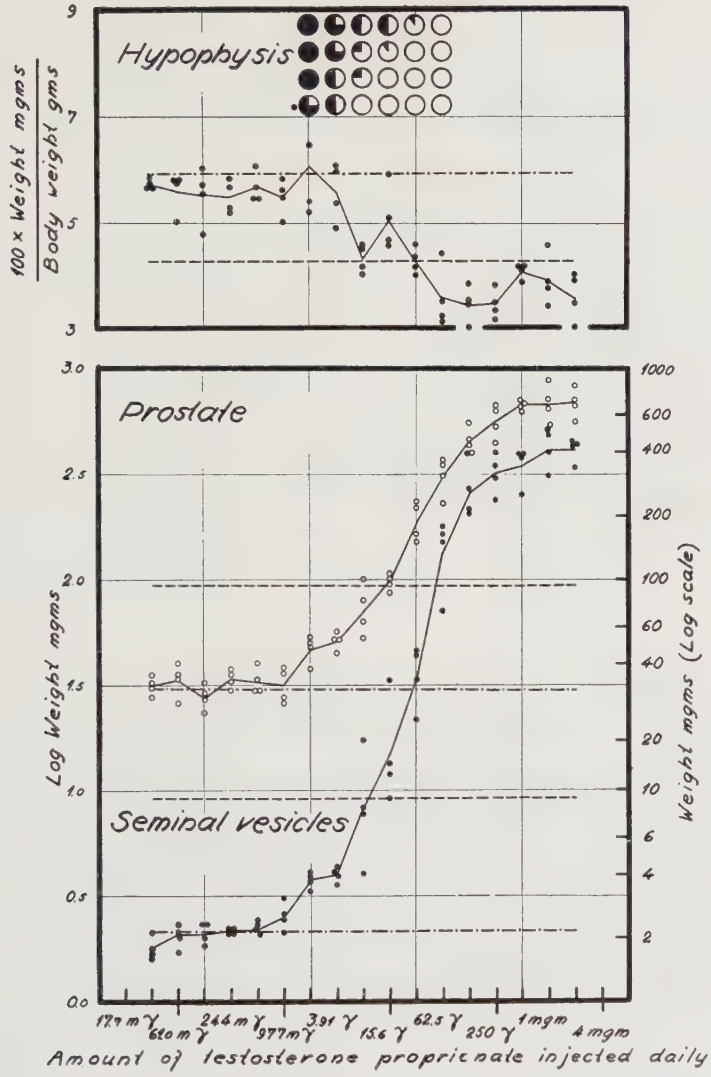


Fig. 28. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with testosterone propionate for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, in the hypophysis chart refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

Scheme A. Preparation of testis extract.

Testis substance is extracted with double the volume 95 % alcohol. 1

Filtrate concentrated to 1/20 vol. (Fract. BI) Shaken with benzone. — Solid ingredients thrown away. 2)

Benzene extract is evaporated to dryness and extracted with acetone at —13° C. Filtered. — Aqueous solution thrown away. 3)

Filtrate evaporated to dryness. Dissolved in petroleum ether. Ether solution shaken with 70 % alcohol. — Insoluble ingredients thrown away. 4)

Alcohol solution evaporated to dryness (Fract. BIV). Dissolved in ethyl ether. Ether solution shaken with 5-N KOH. — Ether solution thrown away. 5)

Ether solution evaporated to dryness (Fract. BVa). — Lye was acidified with HCl using congo-red as indicator and extracted with ethyl ether. Ether solution evaporated to dryness (Fract. BVb). 6)

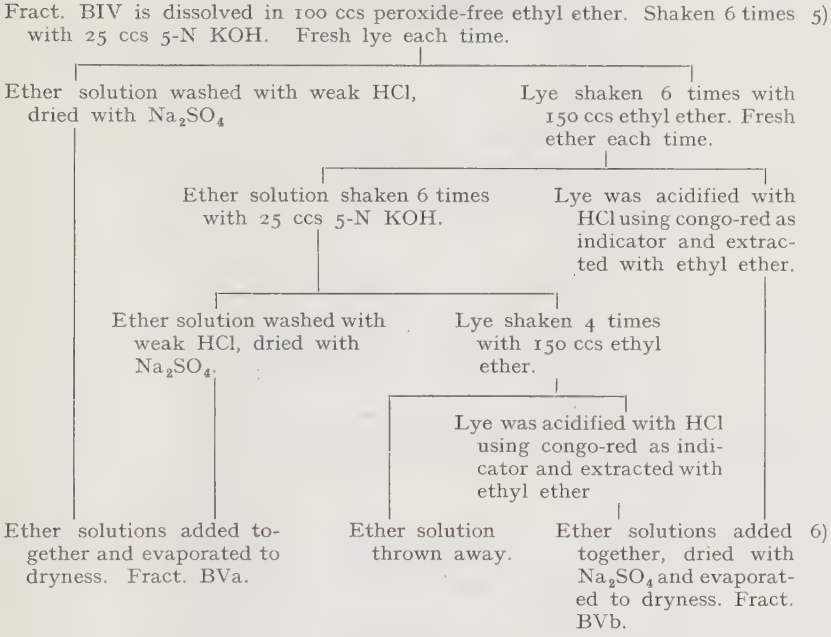
BI correspond to 1 000 gm testis substance. The remainder (4.5 litres) was saturated with sodium chloride and shaken three times with benzene in a machine; fresh benzene was used each time. The aqueous solution was thrown away.

3) The benzene extracts were added together and dried with sodium sulphate. The used sodium sulphate was washed with new benzene. The benzene extract was evaporated to dryness. 1 000 ccs acetone added, and shaken in the shaker. Kept 24 hours at —13° C; filtered. More acetone was added to the insoluble ingredients, which were then shaken in the machine. Kept 24 hours at —13° C; filtered. The insoluble ingredients thrown away.

4) The filtrates were added together and evaporated to dryness. Dissolved in 200 ccs petroleum ether. The ether solution was shaken 3 times with 150 ccs 70 % alcohol, with fresh alcohol each time. The last time shaken in the shaker. The alcohol solutions were added together, and shaken with 100 ccs petroleum ether. This solution was shaken 3 times with 70 % alcohol. The ether solutions were thrown away.

5) The alcohol solutions were added together and concentrated to dryness in a vacuum. Fraction BIV. 1 000 gm testis substance correspond to 931 mg fraction BIV. Dissolved in 100 ccs peroxide-free ethyl ether. Shaken with 5-N KOH according to scheme B, p. 59.

Scheme B. Preparation of testis extract.


Fract. BIV is dissolved in 100 ccs peroxide-free ethyl ether. Shaken 6 times with 25 ccs 5-N KOH. Fresh lye each time. 5)
Ether solution washed with weak HCl, dried with Na_2SO_4
Lye shaken 6 times with 150 ccs ethyl ether. Fresh ether each time.
Ether solution shaken 6 times with 25 ccs 5-N KOH.
Lye was acidified with HCl using congo-red as indicator and extracted with ethyl ether.
Ether solution washed with weak HCl, dried with Na_2SO_4.
Lye shaken 4 times with 150 ccs ethyl ether.
Lye was acidified with HCl using congo-red as indicator and extracted with ethyl ether
Ether solutions added together and evaporated to dryness. Fract. BVa.
Ether solution thrown away.
Ether solutions added together, dried with Na_2SO_4 and evaporated to dryness. Fract. BVb. 6)

6) 1 000 gm testis substance correspond to 35.6 mg fraction BVa, 9.00 mg fraction BVb.

The alcohol extract fraction BI seems to affect both the atrophy of the accessory sex glands and the weight increase of the hypophysis, which sets in after castration (fig. 29). The amount which could be given to the animals was restricted, owing to its being toxic, so that those which got most received 0.10 ccs daily of the non-diluted extract, which correspond to 4.5 gm of testis substance.

When purifying the alcohol extract, great care has been taken so as not to lose any of the activity either on the accessories or on the hypophysis. The effect on the weight increase in the latter is, as has been said earlier, of special interest.

The effect of fraction BIV is shown in fig. 30. A comparison of the fractions BI and BIV shows that the effect on the accessories has not been diminished. The effect on the weight increase of the hypophysis also seems, on the whole, to be unchanged. When dividing the fraction BIV between 5-N KOH and ethyl ether, part of the ingredients passed into the lye (fract. BVb), others into the ether (fract. BVa). The aim with the division was to separate the germinal tissue hormone and testosterone; the latter being wanted free from the former and vice

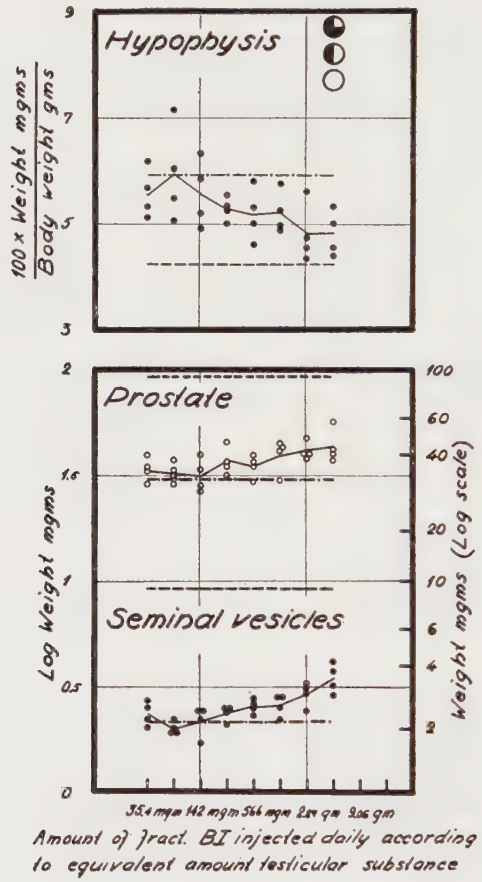


Fig. 29. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. BI for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

versa. It was furthermore desirable that none of the ingredients effecting the hypophysis or accessory sex glands should be lost.

The effect of fraction BVa is shown in fig. 31. A comparison of the fractions BIV and BVa shows that the effect on the accessories has not been diminished. The relation between the effect on the acessories and that on the weight increase of the hypophysis seems to tally, on the whole, with the corresponding relations for testosterone and testosterone propionate.

The effect of fraction BVb is shown in fig. 32. In the doses admini-

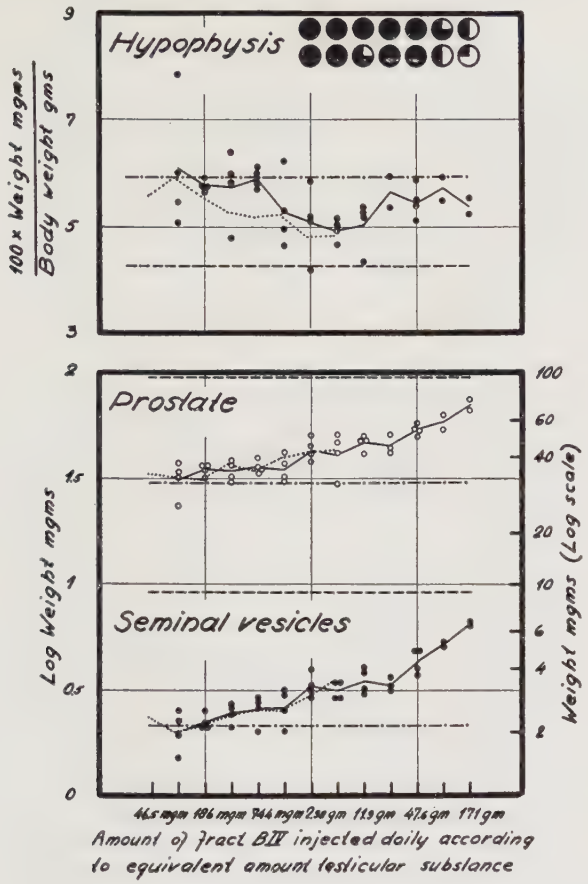


Fig. 30. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. BIV for 3 weeks. ····· Weight, fract. BI. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

stered, it had no effect on the weight of the accessories. On the other hand, it seems to effect the weight increase of the hypophysis, which sets in after castration. It is characteristic that the administration of lesser quantities of both fraction BIV and fraction BVb inhibit the increase of weight. When larger doses are given, the hypophysis weight seems to rise again.

Fraction BVb seems thus to possess the biological qualities which were put forward in the introduction for the hormone of the germinal tissue. This fraction has been further investigated and characterized,

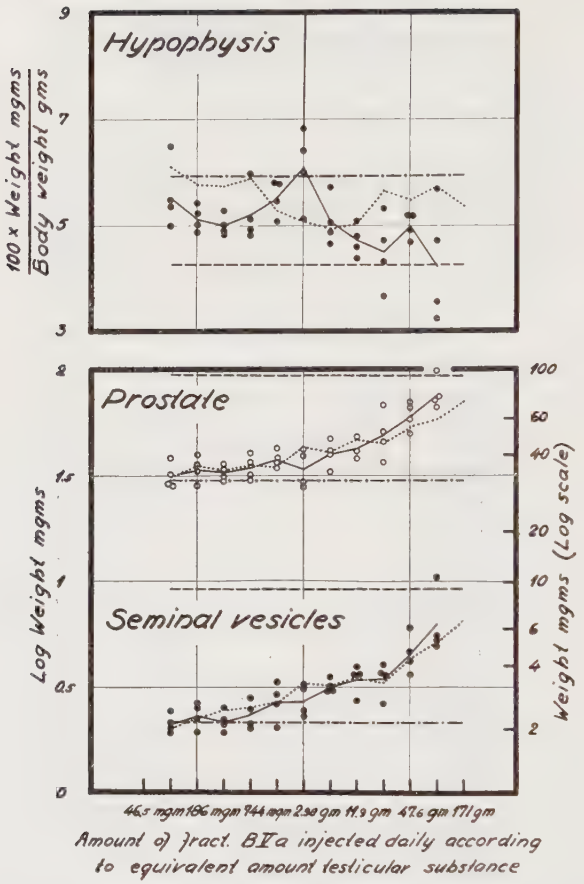


Fig. 31. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. B V a for 3 weeks. ····· Weight, fract. B IV. — — — Weight, normal. —·—·— Weight, castrate.

As the active ingredient was only present in small quantities in the mentioned extracts of bull testes, it has not been possible to continue with this material as basis. Instead, the effect of extracts of boar testes has been investigated.

Extract of boar testes. The extracts of boar testes were prepared in the same way as the extracts of bull testes; here, however, 4 volumes of alcohol were used in the alcohol extraction of the testis substance instead of 2 volumes. The naming of the extracts agrees with that for the extracts of bull testes. 8 kg of testicle substance were first con-

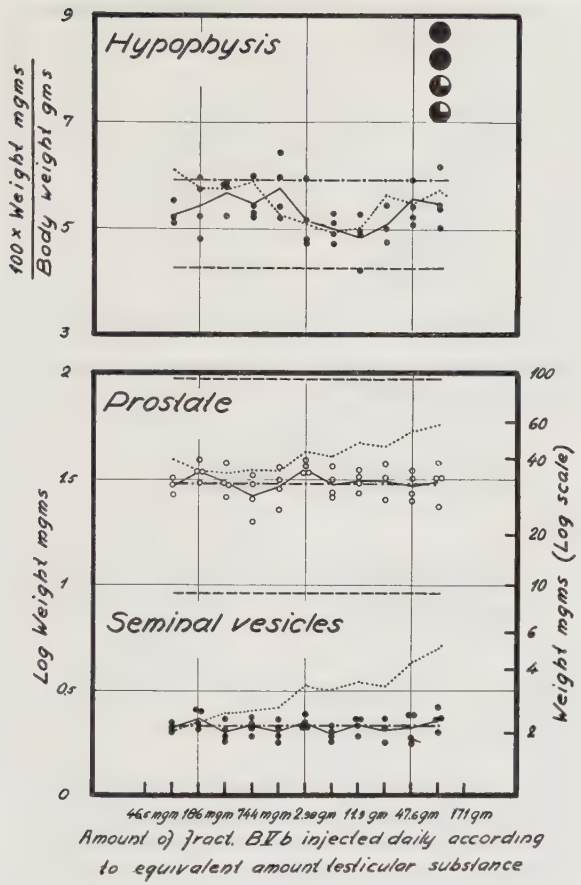


Fig. 32. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. BVb for 3 weeks. ····· Weight, fract. BIV. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

verted; later, 21 kg. The extracts from the earlier preparation were called 1H, from the later one 2H.

The effect of the fractions 1HI and 1HVb is shown in figs. 33 and 34. 1 000 gm testis substance correspond to 52.5 gm fraction 1HI, and 16.2 mg fraction 1HVb. The results agree with those obtained with extract of bull testes. The extracts of boar testes contained considerably more of the active ingredient in the fractions Vb than did the extracts of bull testis.

As it is known that estrogen also occurs in the testicles, the question

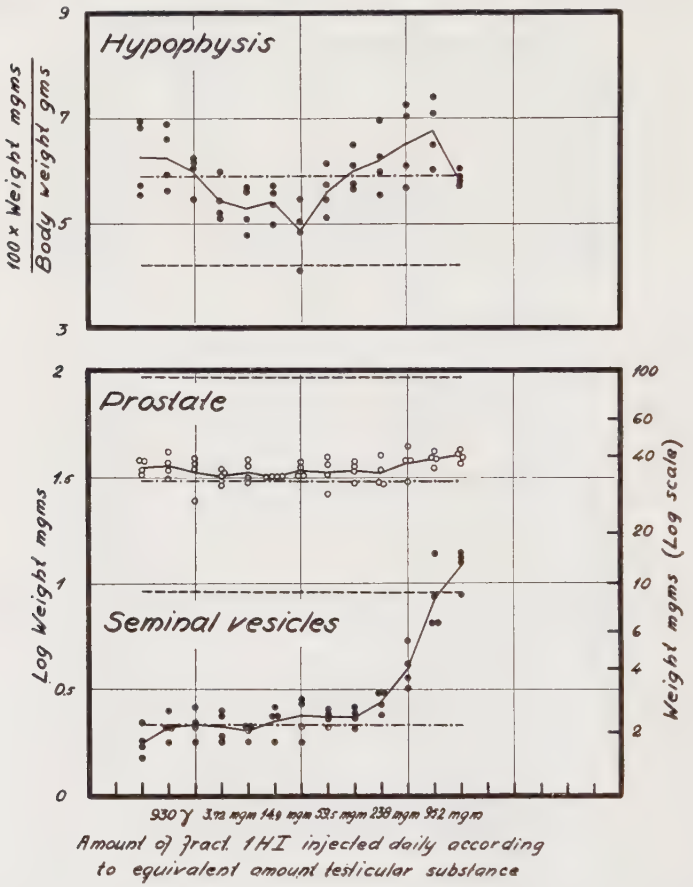


Fig. 33. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. 1 H I for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate.

soon arose whether the active ingredient in the fractions Vb is identical with estrogen. To answer this question, comparative determinations were made of the most important estrogens (estradiol, estrone, estriol) in respect of the following: a) estrous action, b) effect on the weight increase appearing in the hypophysis after castration, c) the effect on the histological castration changes in the hypophysis, d) the effect on the weight of the prostate and seminal vesicles in castrate animals.

Corresponding determinations were also made with fraction 1HVb and also with fractions of 2HVb, where the fractionizing principles tallied with those allowing a chemical separation of the estrogens mentioned.

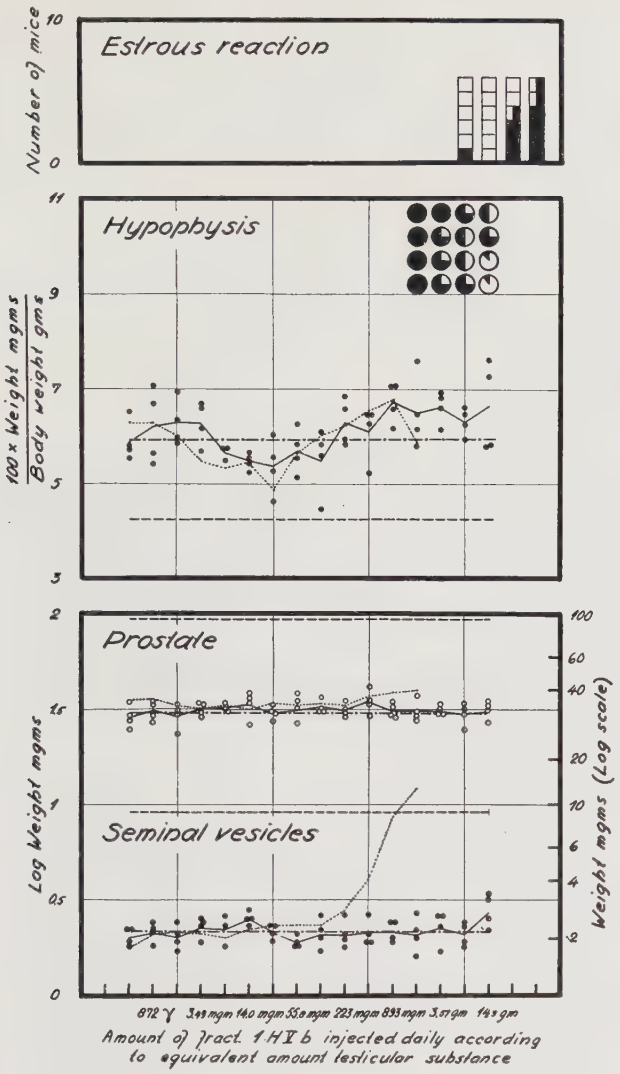


Fig. 34. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. 1 H V b for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

The estrous action was determined on spayed female mice. They were given an injection of 0.10 ccs of the sesame oil solution whose effect is to be investigated. On the 3rd and 4th day after injection, the vaginal smear was examined twice daily, on the 2nd and 5th day once daily. The results are given in the upper diagrams of fig. 34, 35, 37—41. For the mice, the scale at the bottom of the figures shows the dose given once. One MU is the smallest dose which produced estrus in 50 % of the mice treated with the dose in question.

The effect of estradiol, estradiol benzoate, estrone and estriol is shown in figs. 35—38. The fractions BVb and 1HVb, estradiol and estrone, have a similar effect on the weight increase of the hypophysis which sets in after castration. Smaller doses inhibit the increase. When larger doses are given, it rises again. The maximal inhibition occurs with doses as small as about 0.001 MU. The doses mentioned do not effect the appearance of castration cells in the hypophysis, nor yet the weight of the prostate or seminal vesicles. This effect of estradiol and estrone on the increase in the hypophysis weight setting in after castration has not previously been described. As regards estriol, no corresponding effect was observed on the weight increase of the hypophysis. There was no discernible difference between the effect of the estradiol and the estrone on the hypophysis weight. The administration of larger quantities of estradiol, estrone and estriol causes the castration cells in the hypophysis to disappear. Estradiol seems in this respect to be more active than estrone. All three substances affect the weight of the prostate and seminal vesicles; estradiol is in this respect active in smaller doses than is estrone.

Fraction 1HVb proved itself to cause estrus (fig. 34). The relation between the effect on the weight increase of the hypophysis which sets in after castration, and the setting up of estrus, was the same as for estradiol and estrone. There is therefore no reason for assuming that this fraction contains substances which influence the weight increase of the hypophysis but which do not cause estrus.

The chemical nature of the estrogens present in the testicles is not known. Estrone is in all probability present in male urine (Dorfman, Gallagher and Koch 1935).

Methods of separating estradiol, estrone and estriol have been submitted by Smith, Smith and Schiller (1939) and David (1940).

In the further fractionization of the testis extract (fraction 2HVb) it was divided by treatment with semicarbazide into a ketone fraction (estrone) and a non-ketonic fraction (estradiol, estriol). By division between ethyl ether and $^1/_{10}$-N NaOH, the non-ketonic fraction has been divided up into a "weak" phenolic fraction (estradiol) and a "strong" phenolic fraction (estriol). Scheme C, p. 67.

The following method has been used.

6) Fraction 2HVb was added to 33 ccs of an alcoholic solution of

Scheme C. Preparation of testis extract.

Fraction 2HVb is treated with semicarbazide. 6)

Precipitate treated with H_2SO_4. Extracted with ethyl ether. The ether extract evaporated to dryness. Fraction 2HVIa, ketonic.

Soluble ingredients divided between ethyl ether and 1/10-N NaOH 7)

Ether solution evaporated to dryness. Fraction 2HVIIa, non-ketonic, "weak" phenolic.

Lye was acidified with HCl, using congo-red as indicator, and extracted with ethyl ether. Ether solution evaporated to dryness. Fraction 2HVIIb, non ketonic, "strong" phenolic. 8)

semicarbazide acetate (HCl-semicarbazide 3 gm, Na-acetate 3 gm, aqua dest. 20 ccs, 99.5 % ethyl alcohol ad 100 ccs), and boiled, with a condenser, for 3 hours in a water bath. It was evaporated to dryness at 100° C in a vacuum, peroxide-free ethyl ether added 3 times and shaken in a machine. The ether was decanted from the precipitate. Fresh ether was used each time. The ether solutions were added together, evaporated to dryness and added to 33 ccs of the semicarbazide solution. Treated as before.

7) a. The precipitate was added to 33 ccs diluted sulphuric acid (conc. H_2SO_4 15 ccs, aqua dest. 10 ccs, 95 % ethyl alcohol ad 100 ccs). It was boiled, with a condenser, for 3 hours in a water bath. Added together and given 500 ccs of aqua. dest. Shaken 4 times with ethyl ether. The ether solutions were added together and dried with sodium sulphate. Evaporated to dryness. Fraction 2HVIa, ketonic. 1 000 gm testis substance corresponds to 26.9 mg fract. 2HVIa.

b. The ether solutions were added together and shaken 4 times with $^1/_{10}$-N NaOH according to scheme B. Here, emulsions formed, which had to stand up to 10 days to separate.

8) a. The ether fractions were added together and evaporated to dryness. Fraction 2HVIIa, non-ketonic, "weak" phenolic. 1000 gm testis substance correspond to 6.71 mg fraction 2HVIIa.

b. The alcohol fractions were acidified with HCl, using congo-red as indicator, and extracted with ethyl ether. The ether solutions were added together and evaporated to dryness. Fraction 2HVIIb, non-ketonic, "strong" phenolic. 1000 gm testis substance correspond to 23.9 mg fraction 2HVIIb.

The effect of the fractions 2HVIa, 2HVIIa and 2HVIIb is shown in figs. 39—41. The main part of the active substance have passed into the fraction non-ketonic, "weak" phenolic, a lesser part into the

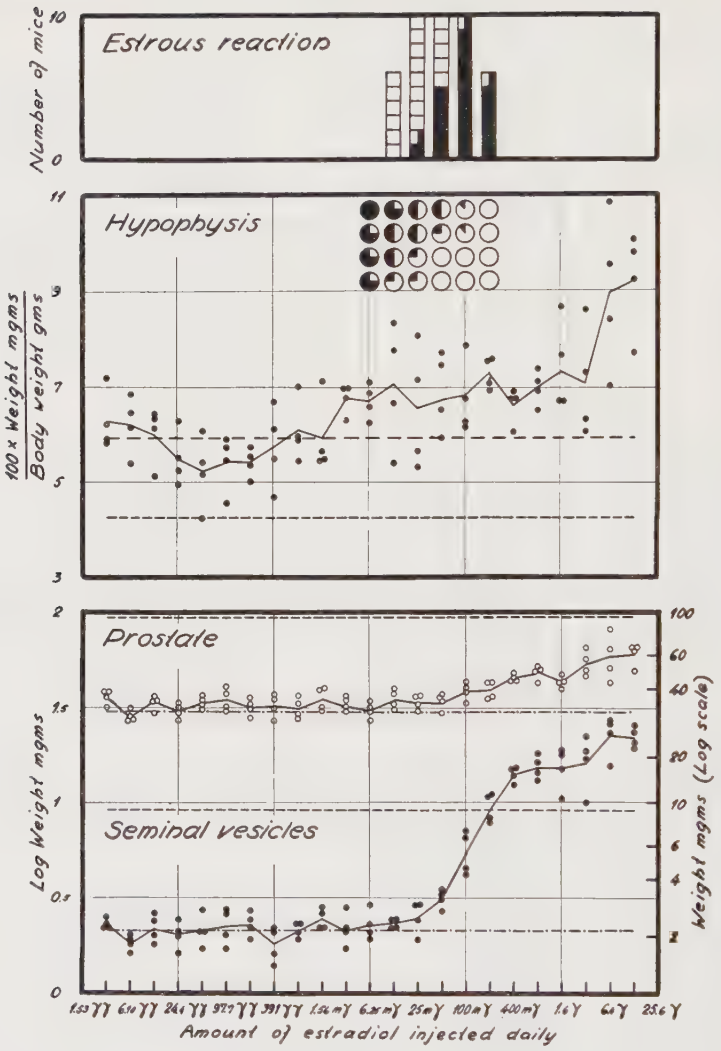


Fig. 35. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with estradiol for 3 weeks. — — — Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

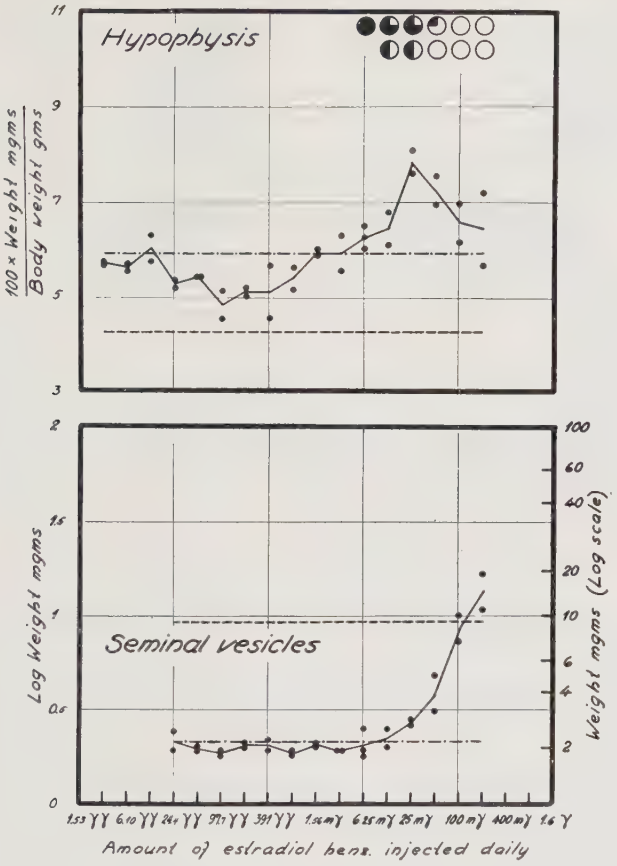

Hypophysis
100 × Weight mgms / Body weight gms
Seminal vesicles
Log Weight mgms
Weight mgms (Log scale)
Amount of estradiol benz. injected daily

Fig. 36. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with estradiol benzoate for 3 weeks. ——— Weight normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

fraction ketonic and only a very small part into the fraction non-ketonic, "strong" phenolic.

The fraction non-ketonic, "weak" phenolic tallies with estradiol as regards all the investigated biological qualities (figs. 42 and 59). As this is so both as regards these properties and also in respect of fractionization, its biologically active substance is in all probability identical with estradiol.

The effect of the fraction ketonic does not seem to agree with that of estrone (fig. 42) as regards the accessory sex glands and the castra-

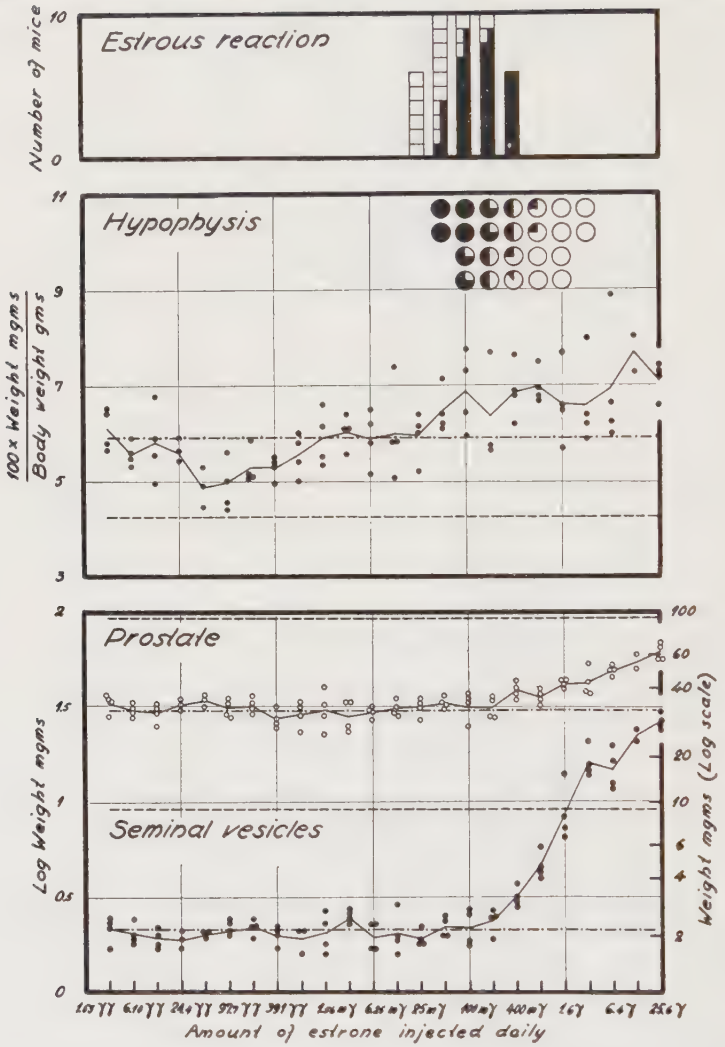


Fig. 37. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with estrone for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

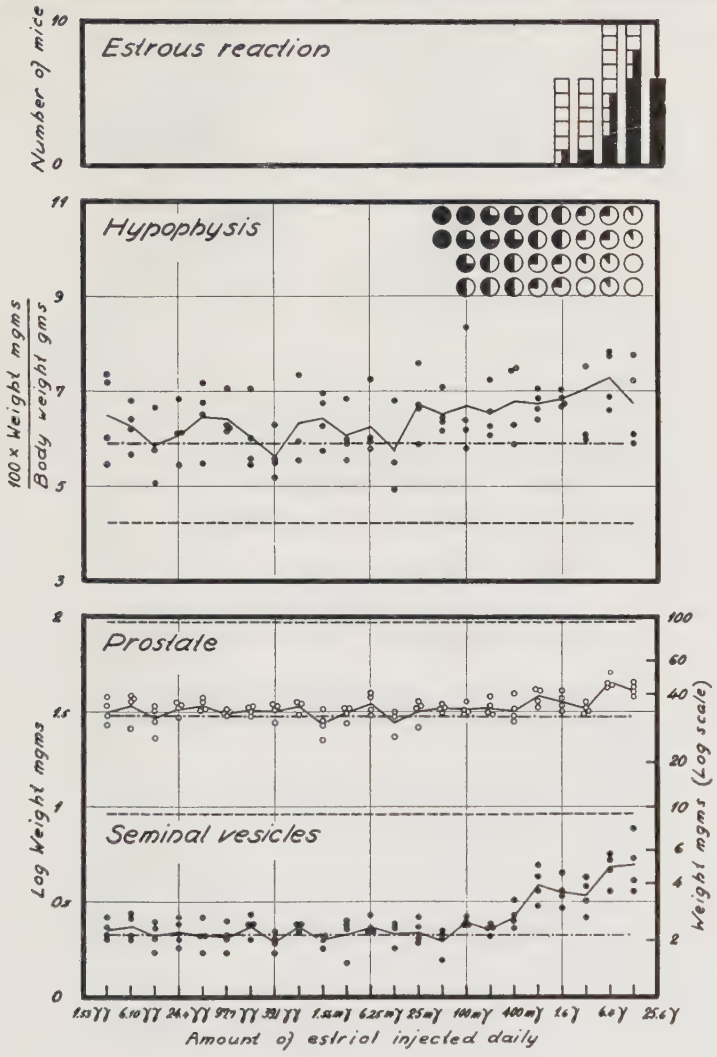


Fig. 38. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with estriol for 3 weeks. ——— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.
In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

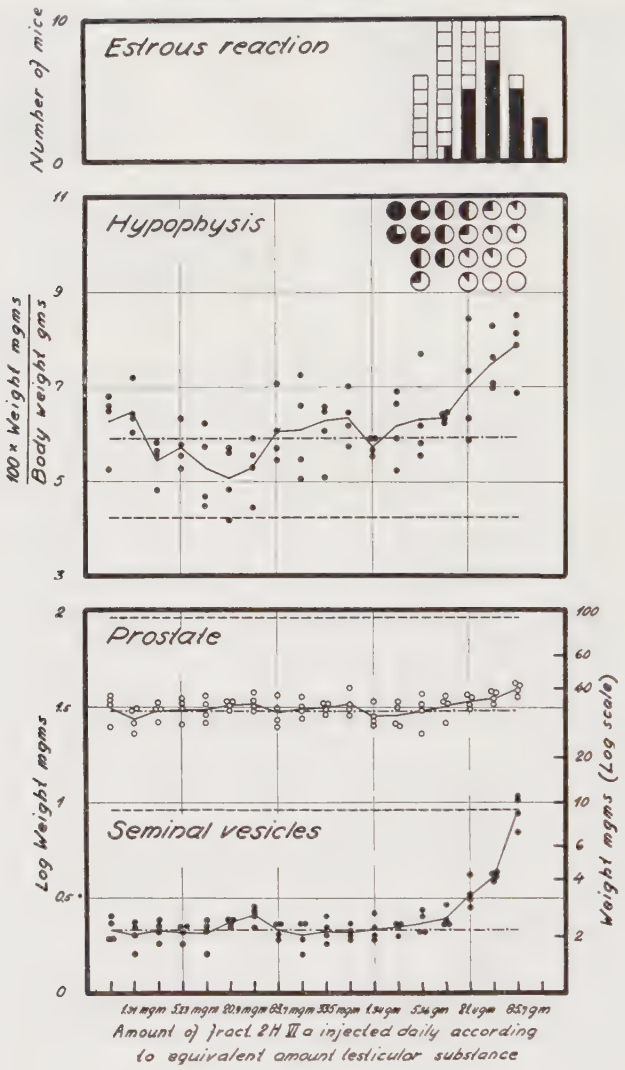


Fig. 39. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. 2 H VI a for 3 weeks. —— Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.
In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

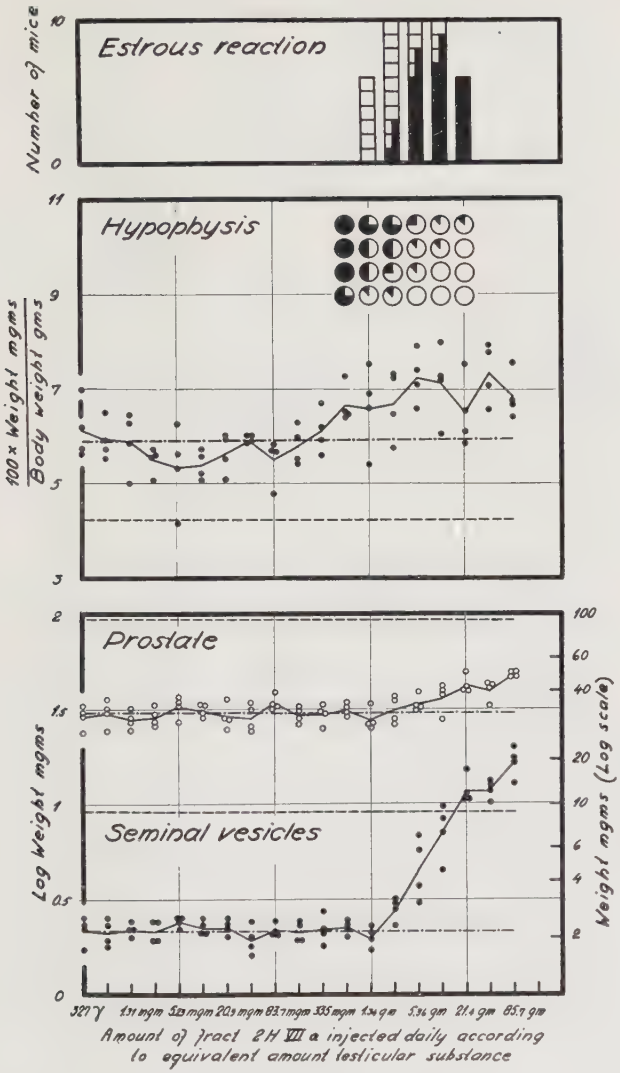

Number of mice
Estrous reaction
Hypophysis
100 × Weight mgms / Body weight gms
Prostate
Seminal vesicles
Log Weight mgms
Weight mgms (Log scale)
327 γ 1.31 mgm 5.23 mgm 20.9 mgm 83.7 mgm 335 mgm 1.34 gm 5.34 gm 21.4 gm 85.7 gm
Amount of fract. 2 H VII a injected daily according to equivalent amount testicular substance

Fig. 40. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. 2 H VII a for 3 weeks. — — Weight, normal. —·—· Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

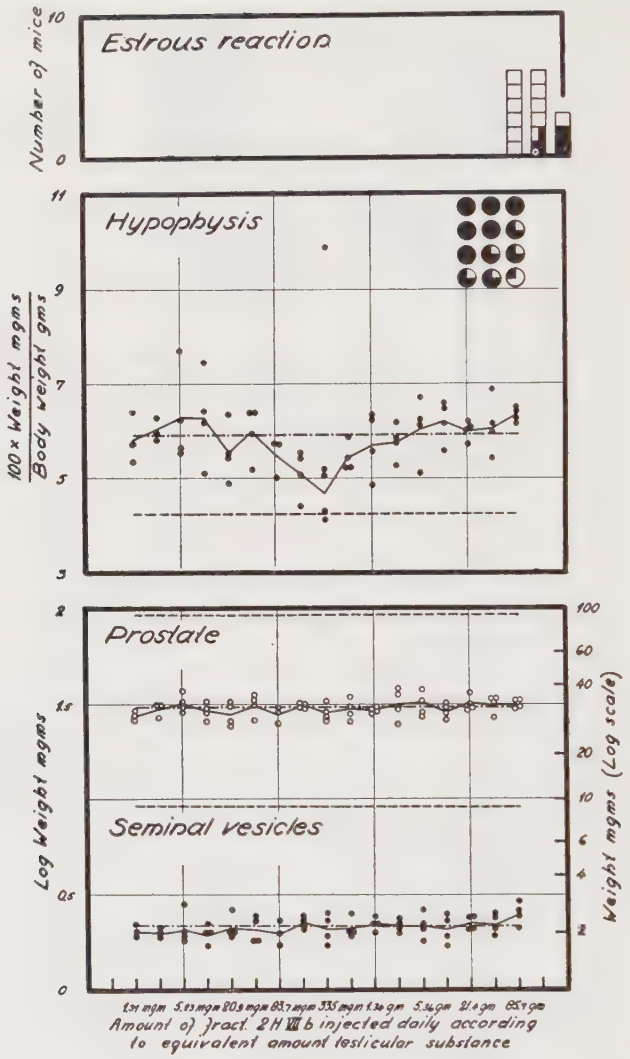


Fig. 41. The two lower charts give the weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with fract. 2 H VII b for 3 weeks. — — — Weight, normal. —·—·— Weight, castrate. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

In the upper chart the height of the columns gives the number of spayed mice injected with the dose. The white part of the column gives the number of mice which did not react with estrus, the black part gives the number of mice which did.

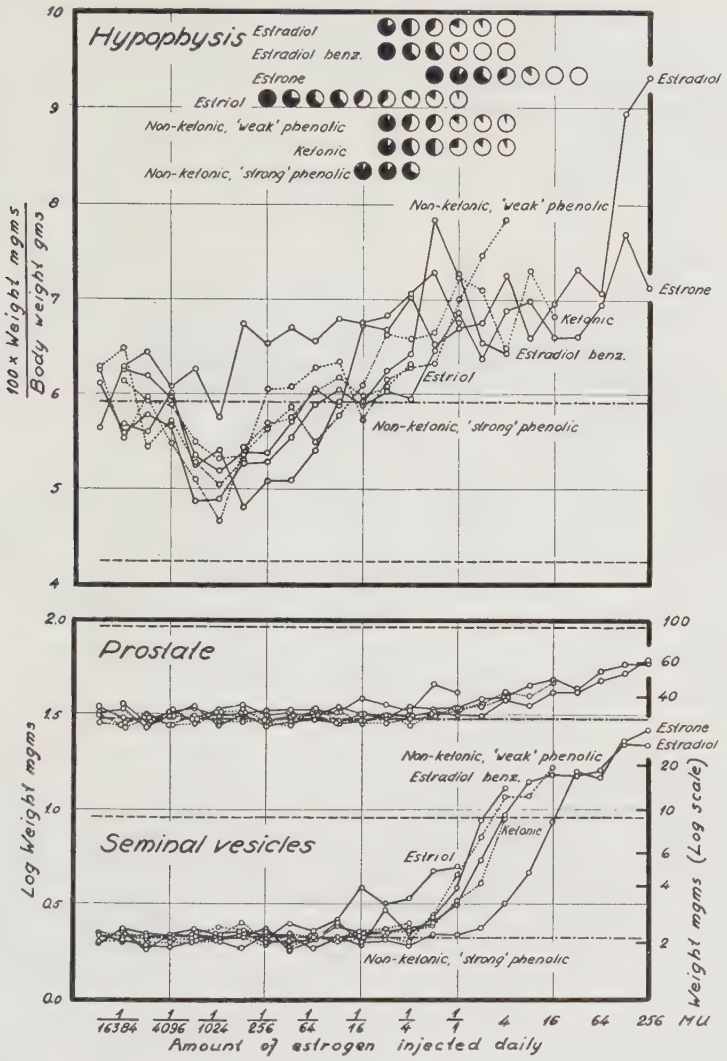


Fig. 42. Weight of hypophysis, prostate and seminal vesicles of castrate infantile rats injected with estradiol, estradiol benzoate, estrone, estriol and fractions 2 H VI a, 2 H VII a, 2 H VII b for 3 weeks. The circles, ○, refer to the occurrence of signet-ring cells. For explanation see p. 18 a. 23.

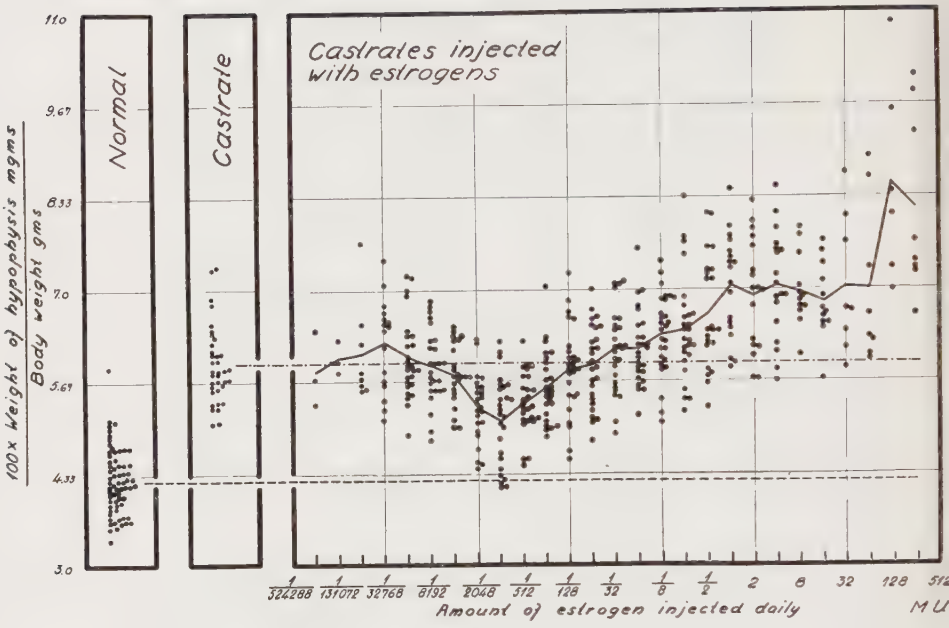


Fig. 43. Weight of hypophysis of normal and castrate infantile rats, and of castrate infantile rats injected with estrogens (estradiol, estradiol benzoate, estrone, and fractions 1 H V b, 2 H VI a, 2 H VII a, 2 H VII b) for 3 weeks.

tion cells in the hypophysis, so that the biologically active ingredient may possibly consist of some ketonic estrogen other than estrone. It does not, however, seem unlikely that the active ingredient in this fraction, too, consists of estradiol. In order to free this fraction from estradiol, the crystalline precipitate obtained from the treatment of fraction 2HVb with semicarbazide, was dried at 100° C in a vacuum, after which it was repeatedly shaken with ethyl ether in a shaking machine. It is possible that the washing with ethyl ether was not able to remove all the estradiol from the precipitate. The effect of the fraction ketonic tallies with that of estradiol (fig. 42).

The fraction non-ketonic, "strong" phenolic was also biologically active. Here, the active ingredient does not seem to be identical with estriol (fig. 42). In this case, too, the effect may be due to estradiol.

In testing the biological activity of these substances and fractions, it has been necessary for practical reasons to restrict the number of experiment animals. It has therefore not been possible in the separate series to make significant the inhibiting effect on the weight increase set up in the hypophysis after castration. The reaction was nonetheless so typical that it could be used in the attempts to isolate the germinal

tissue hormone. To make this effect statistically significant, the material obtained in the administration of estradiol, estrone, and the fractions 1HVb, 2HVIa, 2HVIIa, 2HVIIb have been made into one (fig. 43). All these substances and fractions have been investigated with regard to estrous action, and the series have been lumped together with reference to this action. As tables 6 and 7 show, the inhibition of the weight increase occurring in the hypophysis after castration is statistically significant.

Table 6. *Weight of hypophysis.*

M = mean weight. n = number of animals.

Animals	n	$M \pm \varepsilon(M)$
Normal animals . . . . . (m_1)	66	4.227 ± 0.059
Castrates . . . . . (m_2)	36	5.924 ± 0.092
Castrates + castrates dosed with < 1/4096 MU . (m_3)	118	5.989 ± 0.053
Castrates dosed with 1/2048—1/512 MU . . . . (m_4)	77	5.258 ± 0.061

Table 7. *Difference (D) with reference to weight of hypophysis.*

$D(m_2 - m_1) \pm \varepsilon(D) = +1.697 \pm 0.114$
$D(m_2 - m_4) \pm \varepsilon(D) = +0.666 \pm 0.110$
$D(m_3 - m_4) \pm \varepsilon(D) = +0.731 \pm 0.081$

Summary. A fraction has been isolated from testicles, which, when given to young castrate male rats in small doses, inhibits the weight increase of the hypophysis which sets in after castration; larger doses have the opposite effect. Such doses as inhibit the increase in weight in the hypophysis do not influence that of the prostate and seminal vesicles. This fraction consequently possesses those biological properties which in the introduction were put forward for the hormone of the germinal tissue. The larger part of the biologically active substance in this fraction has proved to tally with estradiol, both as regards fractionization and in respect of tested biological properties. No support has been found for the existence, in testicular extracts, of non-estrogenic substances which possess the biological properties outlined for the germinal tissue hormone.

Chapter 6. Attempts to Substitute the Cessation of the Internal Secretion of the Germinal Tissue by Administering Estrogen.

Introduction and methods. As has been mentioned, a fraction has been isolated from the testicles which showed itself to possess the biological properties given for the hormone of the germinal tissue. The active ingredient in this fraction consists of estrogen. It should therefore be possible to compensate the cessation of the internal secretion of the germinal tissue by administering estrogen. To discover if this be so, estrogen has been given to young male rats which had received the same X-ray treatment as the rats in the series AIXb and BIXb.

When these experiments were begun, the isolation of the germinal tissue hormone had only reached the point where accordance with estrogen had been shown. That with estradiol, shown later, could therefore not be taken into account. — Estrone was used in these substitution tests, chosen because no difference as to the effect on the weight increase set up in the hypophysis after castration was observed in a comparison between the actions of estrone and estradiol. With reference to the effect on the prostate and seminal vesicles, however, estradiol was active in smaller doses than estrone.

Estrone was dissolved in sesame oil. The rats were given a subcutaneous injection of 0.10 ccs daily, receiving the same dose throughout. At the first X-ray treatment, they weighed about 30 gm. They were examined 5 weeks after the first treatment. Hypophysis, prostate and seminal vesicles were prepared and weighed.

The weight of these organs is shown in figs. 44—49 and tables 8 and 9. The weight of those from the animals treated with X-ray and estrone was compared with that of those from normal animals and from X-rayed animals not treated with estrone.

Statistics. *Hypophysis.* In the case of the X-rayed animals which were given estrone, it was also found suitable to reckon with the value for a_1 given in the regression equation for normal animals (16), in order to facilitate the comparison. This simplification was deemed permissible, since in no case was the difference between a_1 in equation (16) and a_1 in the correspondingly calculated equations for the series AXIIIa —AXIIIf larger than 0.9 times the standard error of this difference.

Series AXIIIa.	$y = +0.340 + 0.00306 . x$	(54)
Series AXIIIb.	$y = +0.325 + 0.00306 . x$	(55)
Series AXIIIc.	$y = +0.318 + 0.00306 . x$	(56)

Series AXIIId. $y = +0.304 + 0.00306 . x$ (57)
Series AXIIIe. $y = +0.339 + 0.00306 . x$ (58)
Series AXIIIf. $y = +0.378 + 0.00306 . x$ (59)
y = log hypophysis weight in mg. x = body weight in gm.

The principles given in p. 23 and 25 were used to calculate the difference between the hypophysis weight in the X-rayed animals which had been given estrone and in normal animals, and also in the animals receiving X-ray treatment but not estrone. When the series AIXb and BIXb were made into one, the divergence of the log observed organ weight from the calculated log normal weight for the respective series was made into one.

Prostate and seminal vesicles. As regards the prostate and seminal vesicles, it is characteristic that the administration of smaller doses of estrone inhibits the growth of these organs to a higher degree in small animals than in the larger ones. On this account, the previously mentioned simplification with reference to a_1 has not been made when calculating the following regression equations.

Prostate.	Series AXIIIa.	$y = +0.754 + 0.0131 . x$	(60)
	Series AXIIIb.	$y = +1.199 + 0.00832 . x$	(61)
	Series AXIIIc.	$y = +1.292 + 0.00792 . x$	(62)
	Series AXIIId.	$y = +1.318 + 0.00784 . x$	(63)
	Series AXIIIe.	$y = +1.850 + 0.00327 . x$	(64)
	Series AXIIIf.	$y = +1.716 + 0.00396 . x$	(65)
Seminal vesicles.	Series AXIIIa.	$y = -0.135 + 0.0156 . x$	(66)
	Series AXIIIb.	$y = -0.822 + 0.0183 . x$	(67)
	Series AXIIIc.	$y = -0.380 + 0.0160 . x$	(68)
	Series AXIIId.	$y = -0.417 + 0.0166 . x$	(69)
	Series AXIIIe.	$y = +0.521 + 0.00891 . x$	(70)
	Series AXIIIf.	$y = +0.771 + 0.00532 . x$	(71)

y = log organ weight in mg. x = body weight in gm.

The difference between the weight of prostate and seminal vesicles in the X-rayed animals which received estrone and those which did not has similarly been calculated according to the principles given in p. 23 and 25.

Results and discussion. When estrogen is given to young castrate male rats, the maximal inhibition of the weight increase of the hypophysis occurs at a dose of about 0.001 MU per day. The animals were castrated at a weight of 30 gm, and examined after 3 weeks. As the animals receiving X-ray treatment were not examined until 5 weeks after attaining the weight of 30 gm, there is reason to suppose that they do not react in this way before being given larger quantities of

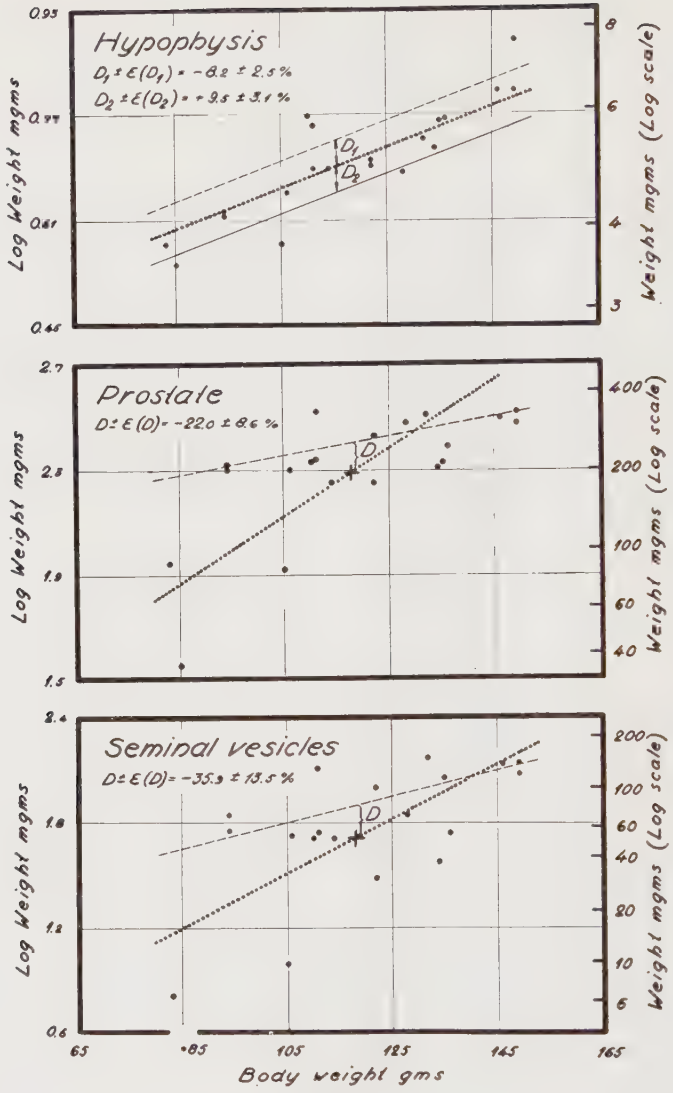

Hypophysis
$D_1 \pm \varepsilon(D_1) = -8.2 \pm 2.5\%$
$D_2 \pm \varepsilon(D_2) = +9.5 \pm 3.1\%$
Log Weight mgms
Weight mgms (Log scale)
Prostate
$D \pm \varepsilon(D) = -22.0 \pm 8.6\%$
Seminal vesicles
$D \pm \varepsilon(D) = -35.9 \pm 13.5\%$
Body weight gms

Fig. 44. Weight of hypophysis, prostate and seminal vesicles of X-rayed, estrone-treated rats, according to body weight. Series A XIII a. X-ray dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. *6,25 mγ estrone daily.* • Observed weight. ····· Calculated weight. Formulas (54), (60) and (66). ——— Calculated normal weight. Formula (16). — — — Calculated weight, X-rayed, not estrone-treated. Formulas (38), (39) and (40).

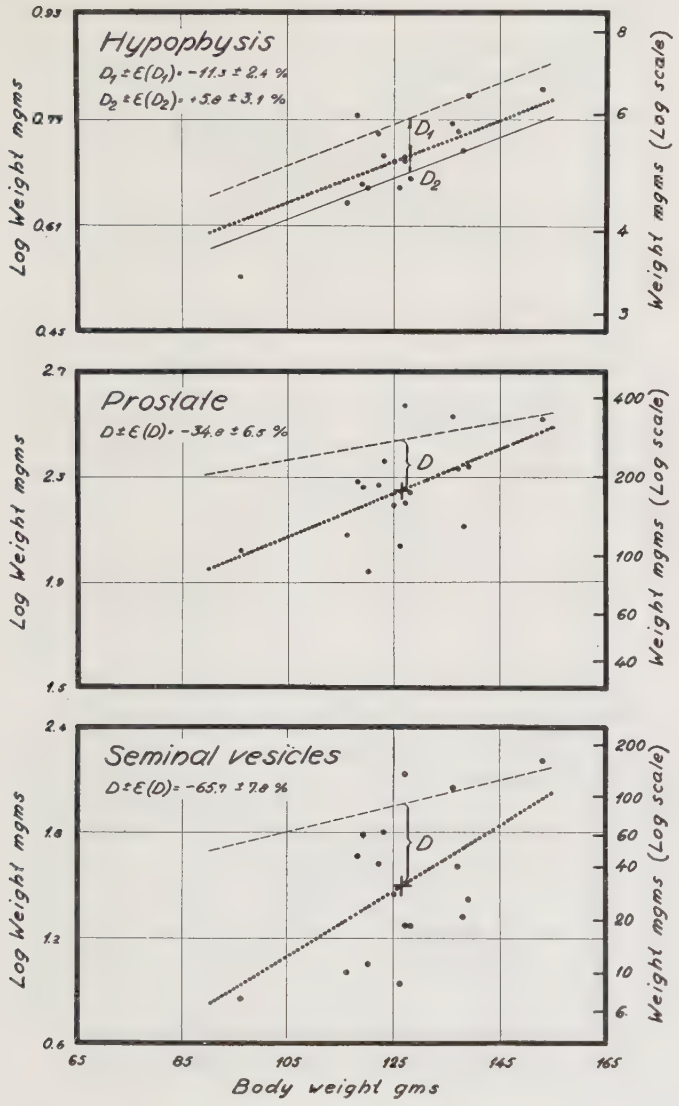


Fig. 45. Weight of hypophysis, prostate and seminal vesicles of X-rayed, estrone-treated rats, according to body weight. Series A XIII b. X-ray dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. *12,5 my estrone daily.* • Observed weight. ····· Calculated weight. Formulas (55), (61) and (67). ——— Calculated normal weight. Formula (16). — — — Calculated weight, X-rayed, not estrone-treated. Formulas (38), (39) and (40).

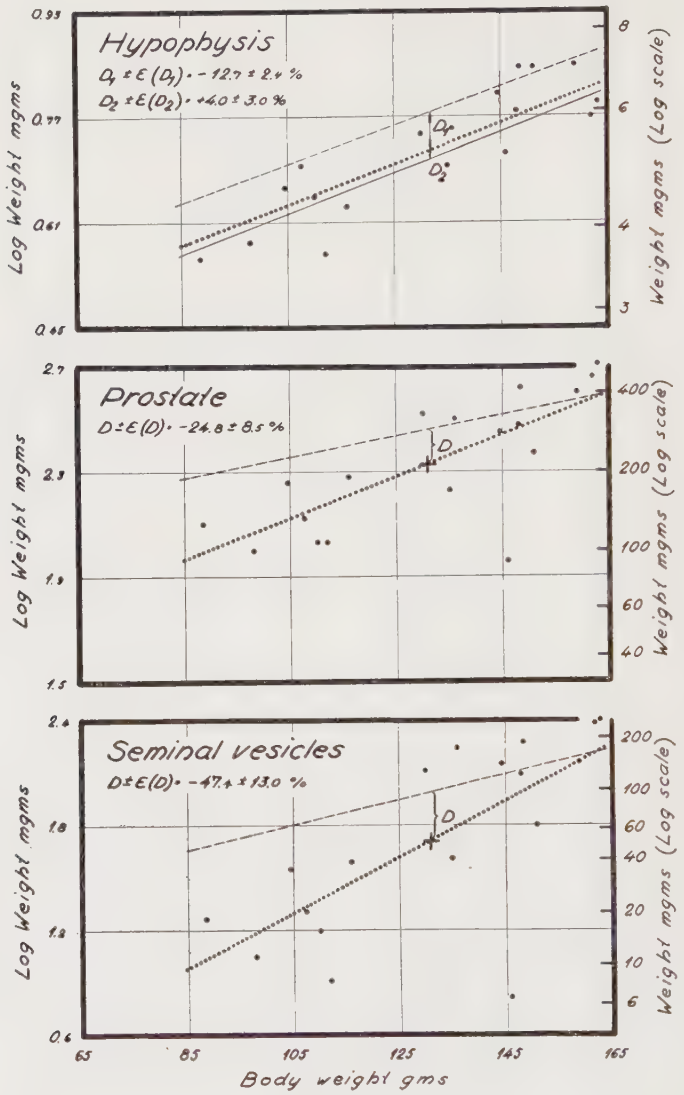

Hypophysis
$D_1 \pm \varepsilon(D_1) = -12.7 \pm 2.4\ \%$
$D_2 \pm \varepsilon(D_2) = +4.0 \pm 3.0\ \%$
Log Weight mgms
Weight mgms (Log scale)
Prostate
$D \pm \varepsilon(D) = -24.8 \pm 8.5\ \%$
Seminal vesicles
$D \pm \varepsilon(D) = -47.4 \pm 13.0\ \%$
Body weight gms

Fig. 46. Weight of hypophysis, prostate and seminal vesicles of X-rayed, estrone-treated rats, according to body weight. Series A XIII c. X-ray dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped *25 my estrone daily*. • Observed weight. ····· Calculated weight. Formulas (56), (62) and (68). ——— Calculated normal weight. Formula (16). — — — Calculated weight, X-rayed, not estrone-treated. Formulas (38), (39) and (40).

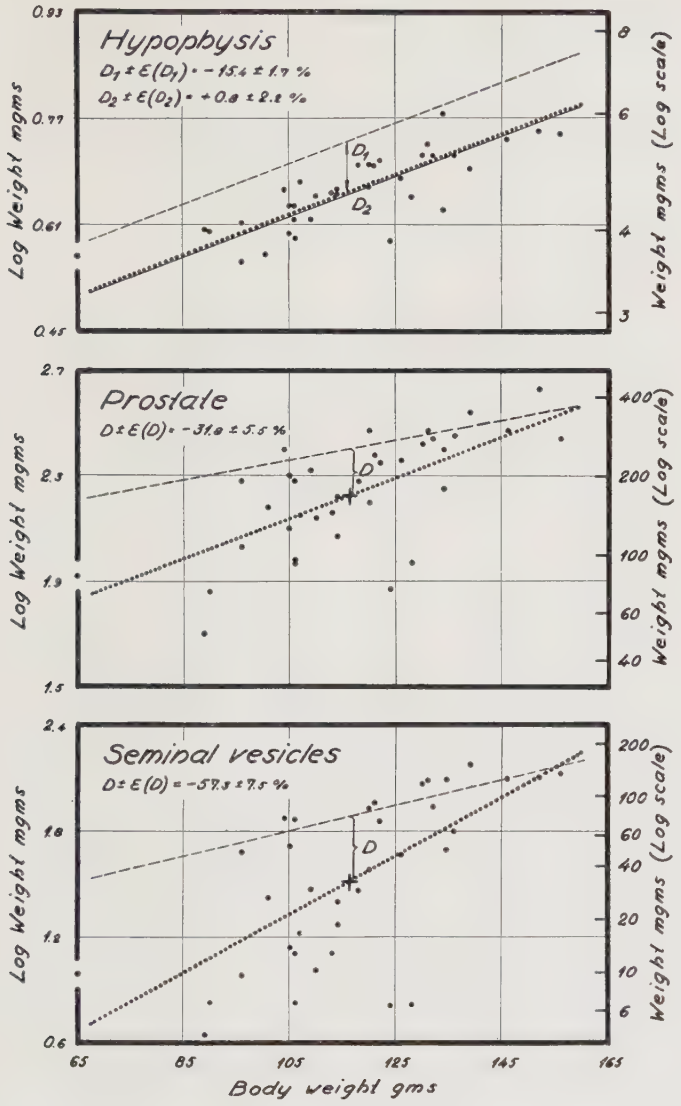


Fig. 47. Weight of hypophysis, prostate and seminal vesicles of X-rayed, estrone-treated rats, according to body weight. Series A XIII d. X-ray dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. *50 my estrone daily.* • Observed weight. ····· Calculated weight. Formulas (57), (63) and (69). ——— Calculated normal weight. Formula (16). — — — Calculated weight, X-rayed, not estrone-treated. Formulas (38), (39) and (40).

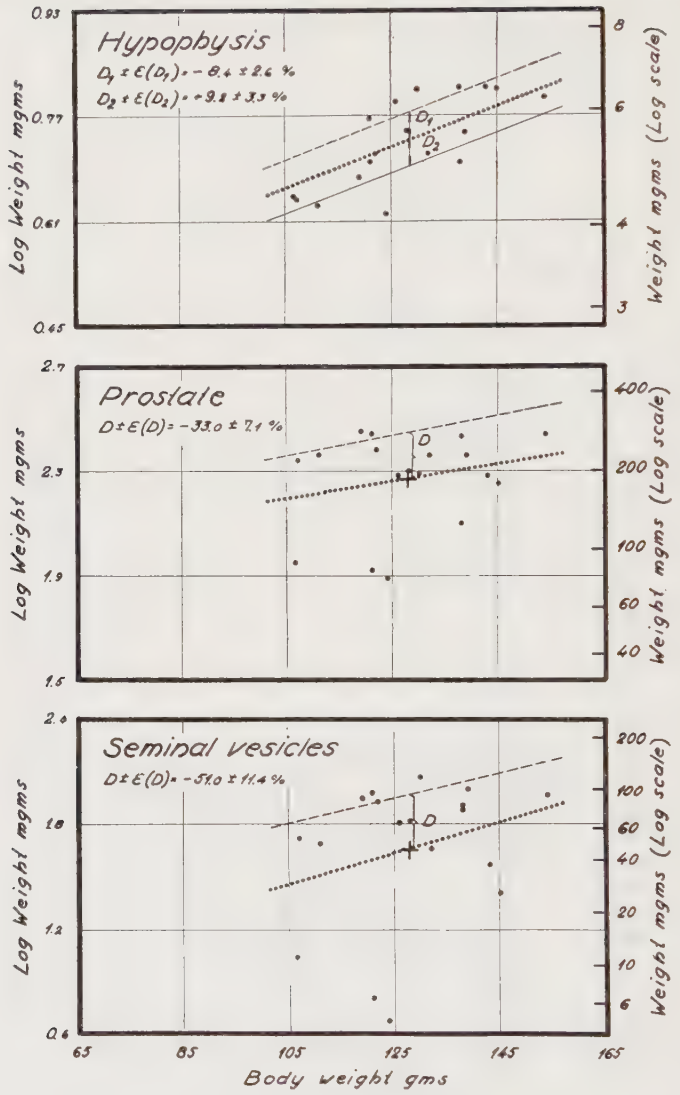

Hypophysis
D1 ± E(D1) = −8.4 ± 2.6 %
D2 ± E(D2) = +9.8 ± 3.3 %
Log Weight mgms
Weight mgms (Log scale)
Prostate
D ± E(D) = −33.0 ± 7.1 %
Seminal vesicles
D ± E(D) = −51.0 ± 11.4 %
Body weight gms

Fig. 48. Weight of hypophysis, prostate and seminal vesicles of X-rayed, estrone-treated rats, according to body weight. Series A XIII e. X-ray dose 233 + 117 = 350 r. Time of exposure 20 min. Circulation stopped. *100 my estrone daily.* • Observed weight. ····· Calculated weight. Formulas (58), (64) and (70). ——— Calculated normal weight. Formula (16). — — — Calculated weight, X-rayed, not estrone-treated. Formulas (38), (39) and (40).

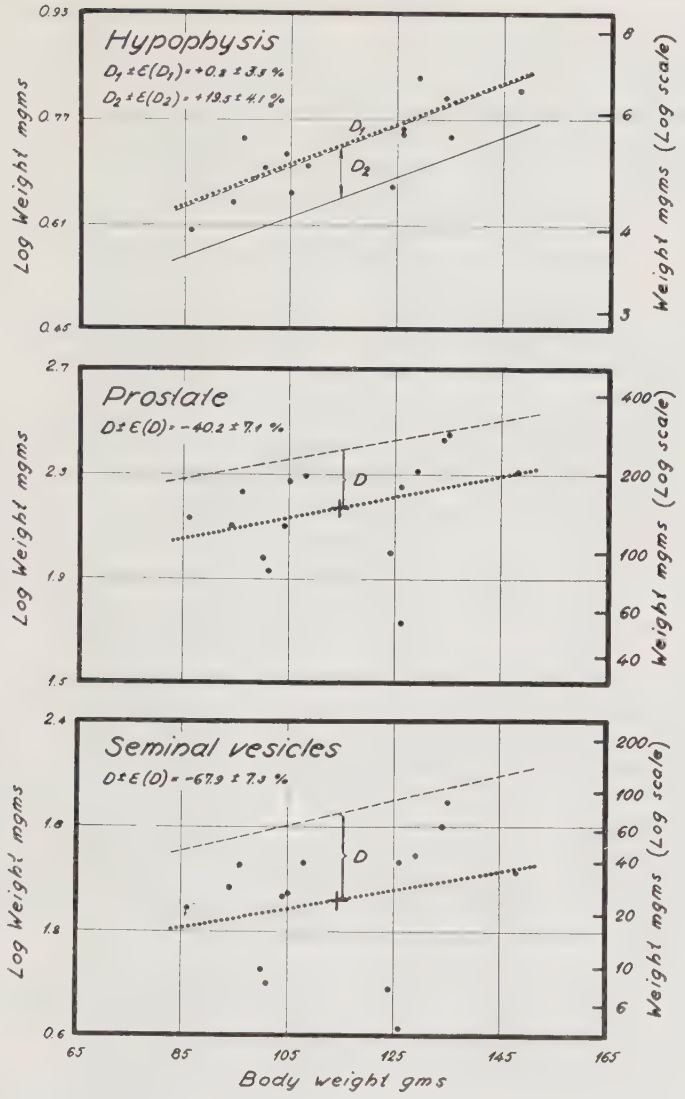


Fig. 49. Weight of hypophysis, prostate and seminal vesicles of X-rayed, estrone-treated rats, according to body weight. Series A XIII f. X-ray dose 233 + 117 = 350 r Time of exposure 20 min. Circulation stopped. *400 my estrone daily.* • Observed weight. ····· Calculated weight. Formulas (59), (65) and (71). ——— Calculated normal weight. Formula (16). — — — Calculated weight, X-rayed, not estrone-treated. Formulas (38), (39) and (40).

estrogen since, there is probably a very great increase in the production of the germinal tissue's hormone during this period of time, as in the production of the hormone stimulating the growth of the prostate and the seminal vesicles (cf. fig. 2).

As is shown in figs. 44—49 and tables 8—9, p. 87 a. 88, it is possible by the administration of estrone to prevent the weight increase set up in the hypophyses of the animals treated with X-ray. As was supposed, however, daily doses considerably larger than 0.001 MU had to be used. A daily dose of 50 mγ estrone gave the best agreement with the normal animals. The effect on the hypophysis weight tallies with the one observed in administration of estrogen to castrate animals. When the doses are increased, the weight increase is at first inhibited and then rises again.

It has been shown earlier on in this work that a stopping of the blood circulation through the testicles during the X-ray treatment intensifies the weight increase set up in the hypophyses of animals receiving this treatment. At the same time there is an increase in the production of the hormone stimulating the growth of the prostate and seminal vesicles. The reason for this increased production has been discussed, and it is asserted to be released by the cessation of the internal secretion of the germinal tissue via the hypophysis. That this really is the case is shown in the inhibited growth of the prostate and the seminal vesicles following a dose of estrogen sufficiently large to prevent the weight increase in the hypophysis. In smaller doses, too, the difference between the weight of prostate and seminal vesicles in the X-rayed animals which have, and those which have not, received estrogen, is statistically significant. The growth inhibition of prostate and seminal vesicles is then particularly marked in the smaller animals. This is explained partly by the fact that the smaller animals receive more estrogen per gm body weight, partly because in all probability the normal production of the germinal tissue hormone is less in little animals, so that they do not need so much estrogen to compensate the cessation of this internal secretion.

Summary. The administration of estrogen makes it possible to prevent the weight increase of the hypophysis setting in on the cessation of the internal secretion of the germinal tissue.

It has been asserted in a previous chapter that the cessation of the germinal tissue's internal secretion via the hypophysis releases a greater formation of the testicle hormone stimulating the growth of the accessory sex glands. This is proved to be true by the fact that, if the changes set up in the hypophysis on the cessation of the germinal tissue's internal secretion are prevented from appearing by the administration of estrogen, there is no increased production of the testicle hormone mentioned.

Table 8. *Difference (D) between weight of hypophysis in rats with X-ray-treated testicles, dosed with estrone, and in normal animals (series AI, 51 animals, and BI, 20 animals). The difference is expressed in % of the normal weight. The sign + shows increase, the sign — decrease. The difference is given in italics if it is equal to or greater than 3 times the standard error of the difference.*

Amount of estrone given daily	Total dose	No. of X-ray treatments	Ratio of size of the doses	Time of exposure in each X-ray treatment	Circulation	Hypophysis $D \pm \varepsilon(D)$
0 mγ. Ser. AIX b (31 animals)	350 r	2	2 : 1	20 mins.	Stopped 30 mins.	*+19.3±2.6 %*
0 mγ. Ser. BIX b (18 animals)	»	»	»	»	»	*+21.1±2.9 %*
0 mγ. Ser. AIX b + BIX b (49 animals)	»	»	»	»	»	*+19.9±2.4 %*
6.25 mγ. Ser. AXIII a (20 animals)	»	»	»	»	»	*+ 9.5±3.1 %*
12.5 mγ. Ser. AXIII b (17 animals)	»	»	»	»	»	+ 5.8±3.1 %
25 mγ. Ser. AXIII c (18 animals)	»	»	»	»	»	+ 4.0±3.0 %
50 mγ. Ser. AXIII d (36 animals)	»	»	»	»	»	+ 0.8±2.2 %
100 mγ. Ser. AXIII e (18 animals)	»	»	»	»	»	+ 9.2±3.3 %
400 mγ. Ser. AXIII f (15 animals)	»	»	»	»	»	*+19.5±4.1 %*

Table 9. *Difference (D) between the weight of hypophysis, prostate and seminal vesicles in X-ray-treated rats dosed with estrone and in X-ray-treated rats not dosed with estrone (series AIX b and AIX b + BIX b). The difference is expressed in % of the weight in roentgen-treated rats not dosed with estrone. The sign + shows increase, the sign — decrease. The difference is given in italics if it is equal to or greater than 3 times the standard error of the difference.*

Amount of estrone given daily	Hypophysis		Prostate		Seminal vesicles	
	Series AIX b (31 animals) D ± ε (D)	Series AIX b + BIX b (49 animals) D ± ε (D)	Series AIX b (31 animals) D ± ε (D)	Series AIX b + BIX b (49 animals) D ± ε (D)	Series AIX b (31 animals) D ± ε (D)	Series AIX b + BIX b (49 animals) D ± ε (D)
6.25 mγ. Ser. AXIII a (20 animals)	*— 8.2 ± 2.5 %*	*— 8.7 ± 2.3 %*	*— 22.0 ± 8.6 %*	*— 21.1 ± 8.4 %*	*35.9 ± 13.5 %*	*— 32.8 ± 13.5 %*
12.5 mγ. Ser. AXIII b (17 animals)	*— 11.3 ± 2.4 %*	*— 11.8 ± 2.3 %*	*— 34.8 ± 6.5 %*	*— 34.1 ± 6.3 %*	*65.7 ± 7.8 %*	*64.0 ± 7.8 %*
25 mγ. Ser. AXIII c (18 animals)	*— 12.7 ± 2.4 %*	*— 13.2 ± 2.2 %*	*— 24.8 ± 8.5 %*	*— 24.0 ± 8.3 %*	*47.4 ± 13.0 %*	*— 41.8 ± 13.2 %*
50 mγ. Ser. AXIII d (36 animals)	*— 15.4 ± 1.7 %*	*— 15.9 ± 1.5 %*	*— 31.8 ± 5.5 %*	*— 31.0 ± 5.2 %*	*— 57.3 ± 7.5 %*	*— 55.2 ± 7.3 %*
100 mγ. Ser. AXIII e (18 animals)	*— 8.4 ± 2.6 %*	*— 8.9 ± 2.5 %*	*— 33.0 ± 7.1 %*	*— 32.2 ± 6.9 %*	*— 51.0 ± 11.4 %*	*— 48.0 ± 11.0 %*
400 mγ. Ser. AXIII f (15 animals)	*+ 0.2 ± 3.3 %*	*— 0.4 ± 3.1 %*	*— 40.2 ± 7.1 %*	*— 39.5 ± 6.9 %*	*— 67.0 ± 7.3 %*	*— 66.4 ± 7.4 %*

Chapter 7. Attempts to Show Direct the Increased Production of Gonadotropic Hormone Setting in on the Cessation of the Internal Secretion of the Germinal Tissue.

It has been seen in the earlier account that a cessation of the internal secretion of the germinal tissue releases a heightened production of gonadotropic hormone in the hypophysis.

Attempts have been made to show direct the increased quantity of gonadotropic hormone in the blood. A particular method has been worked out to this end.

Administration of gonadotropic hormone to infantile females causes maturing of the ovaries with formation of estrogen. Infantile ovaries were implanted intrarenally in the animals to be investigated for the content of gonadotropic hormone in the blood, in the hope that they, too, would react in the same way as stated above to an increase of gonadotropic hormone. When such implantation is carried out on castrate male animals, later histological investigation showed that ripening of the follicles really does take place. The follicle ripening in the implanted ovaries was nevertheless not sufficiently typical and regular to be suitable as a test.

The male rats were castrated at a weight of 30 gm. The implantation was made 3 weeks after castration, the ovaries being taken from female rats weighing about 30 gm. One ovary was implanted in each kidney. The implanted ovaries were prepared and histologically examined 2 weeks after implantation.

When infantile females are given gonadotropic hormone, the ripening of the follicles is accompanied by an increased formation of estrogen. Vaginal pieces from infantile females were at the same time implanted intramuscularly in the male rats to discover whether implanted ovaries would also react in this way, and in the hope that the increased production of estrogen would be easier to prove than the follicle ripening — preparatory tests having shown that vaginal pieces implanted intramuscularly react with typical estrus if the animal is given estrogen. Six pieces were implanted in each male rat, two in the gluteal musculature on each side and one in the thigh muscles. These implantations were carried out at the same time as those of the ovaries. Vaginal pieces and ovaries in each case were from the same female. The vaginal pieces were extirpated, one or two every day, or every other day, beginning with the 7th day after implantation. As table 10 shows, in all the castrate males (11 animals) estrus reaction occurred in one or more of the implanted vaginal sections. This was never so in normal male rats (12 animals) implanted with ovaries and vaginal

Table 10. *Appearance of epithel squamae in implanted pieces of vagina.*

Group	Animal	Day after implantation					
		7th	8th	9th	11th	13th	15th
Castrates	1	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
	2	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +
	3	+ + +	+ + + +	+ + + +	+ + +	+ + + +	+ + + +
	4	+ +	+ + + +	+ + + +	+ + +	+ + + +	
	5	(+)	(+)	+	+ + + +		
	6	—	—	+ +	+ +	+ + + +	+ +
	7	+ + + +	+ + + +	+ + + +	+ + + +	+ + +	
	8	+	—	+ + +	+	(+)	(+
	9	(+)	(+)	+	+ + +	+ + + +	+ + + +
	10	+ + +	+ + + +	+ + + +	+ +	+	+ + + +
	11	+ + +	+ + + +	+ + +	+ + +	+ + +	+ + + +

pieces in corresponding wise. It is thus possible with this process to show the increased amount of gonadotropic hormone in castrate male rats. When ovaries and vaginal pieces were implanted in male rats (12 animals) which had received the same X-ray treatment as the animals in Series AIXb and BIXb, no unequivocal estrous reaction could ever be shown in the vaginal pieces. The implantations were made 3 weeks after the first X-ray treatment. Thus the increased production of gonadotropic hormone released by the X-ray treatment does not seem to be as great as that set up by castration.

As the production of the gonadotropic hormone is inhibited in the hypophysis both by the hormone formed in the germinal tissue and by that formed in the interstitial cells, it is naturally not so stimulated by a cessation of the internal secretion of the former as it is by castration.

The results are of interest in the interpretation of the investigations by Steinach (1912, 1916), Sand (1918) and Lipschütz (1925, 1926) into the feminizing ovary implantation in male guinea-pigs. The implantation of ovaries in a castrate guinea-pig has a feminizing effect on its teats; the same effect is observed in animals with experimental cryptorchism. In normal animals, the feminizing effect is only produced from intratesticular implantation, and then only in about 20 % of the animals operated, according to Sand. These observations have given rise to several hypotheses, including Steinach's theory of the antagonism between the sex hormones.

In my opinion, it should be quite clear that the positive results on castrate males and males with experimental cryptorchism is due to the fact that when the ovaries are implanted, they are exposed to a

more enhanced action of gonadotropic hormone than they would be in normal animals. When the investigations cited above were made, the gonadotropic hormone of the hypophysis was not known, so there were no premises for the explanation now given.

According to LIPSCHÜTZ, the inhibitory effect of the testicles on the feminizing action disappears not only on castration but also in suppressed spermatogenesis. As regards castration, he considers the action to depend on the cessation of the testicular internal secretion. The effect of suppressed spermatogenesis is not, however, interpreted as being due to the cessation of an internal secretion of this tissue. Instead he attributes it to disturbance of this function liberating sensitizing substances which are thought to be formed in the seminiferous tubules on the degeneration of the germinal epithelium, and which pass into the blood. As LIPSCHÜTZ pointed out, the fact that a feminizing effect sometimes occurs when ovaries are intratesticularly implanted in otherwise normal males is probably due to the operation injuring the spermatogenesis.

Summary. A new method is described for showing an increased production of gonadotropic hormone. The principle is that an increased formation of this hormone sets up follicle ripening in infantile ovaries implanted intrarenally, and secundary estrous reaction in vaginal pieces implanted intramuscularly. By means of this method it is possible to show the increased production of gonadotropic hormone which sets in after castration. The increased production occurring in isolated cessation of the germinal tissue's internal secretion could not, on the other hand, be shown by means of this method.

The investigations of STEINACH, SAND and LIPSCHÜTZ into feminizing ovary implantations in male guinea-pigs are discussed.

Chapter 8. Attempts to Induce Prostatic Hypertrophy in Dogs by Administering Testosterone Propionate.

Introduction. By showing that cessation of the internal secretion of the germinal tissue via the hypophysis sets up an increased production of the testicle hormone stimulating the growth of prostate and seminal vesicles, the question is brought to the fore: Is hypertrophy of the human prostate due to a corresponding functional disturbance?

Dogs were treated with testosterone propionate to investigate whether an increased action of testosterone produces prostatic hypertrophy. These animals were chosen for the experiments since the structure of their prostate glands agrees with that of the human being; also this gland is often enlarged in older dogs, causing difficulty in

urination. The histological appearance of the prostate in dogs with hypertrophy of this organ has been described by KRACHT-PALÉJEFF (1911), KOTTMAN (1935), ZUCKERMAN and GROOME (1937) and ZAHLER (1939). They report now a diffuse hyperplasia of the gland, often with large cysts, now a nodular one. The nodules appear as a rule to come from the periurethral glandular tissue. It is difficult to form an idea of the frequency of the different types, since they are not sharply marked off from one another, and since a number of investigators have only observed the nodular type, others only the diffuse type, and still others have observed both types. ZUCKERMAN and GROOME (1937) report the height of the glandular epithelium to be increased.

The effect on dog prostate of an administration of testosterone has earlier been investigated by RÖSSLE and ZAHLER (1938) and also by GAARENSTROOM, DE JONGH and KOK (1940). The former gave one dog 5 mg testosterone per day for 15 days, another dog 25 mg testosterone propionate per day for 25 days. The first dog died after 15 days on account of retention of urine. When the prostates from both dogs were examined, they showed a strong, diffuse hyperplasia. According to ZAHLER (1939), the periurethral glands do not take part in the enlargement. GAARENSTROOM and co-workers did not observe any enlargement of the prostate after administering testosterone propionate. The quantities they gave were, however, smaller.

Methods. In order to obtain a material as homogeneous as possible the experiments only used dogs of the same race, *i. e.* harriers, and of about the same age and size. The amount of testosterone propionate given varied between 5 and 125 mg per day, and the time of the treatment between 36 and 118 days. The testosterone propionate was dissolved in sesame oil and injected subcutaneously. The animals were killed at the end of the treatment; the prostate and bladder prepared and weighed. The prostate was examined histologically. Fixation: 10 % formalin. Staining: haematoxilin-eosin.

Results and discussion. Table 12, p. 93, gives the age, weight and shoulder height of the dogs at the end of the treatment, and also the weight of prostate and bladder. Table 11, p. 93, gives the corresponding values for the normal animals.

As fig. 50 shows, the administration of 5 mg testosterone propionate per day causes no sure increase of weight in the prostate. When the dose is increased to 25 or 125 mg per day, a distinct enlargement of the gland is set up. Difficulties in urination were displayed after about 2 months by the animals receiving 25 mg per day, and after about 1 month by those receiving 125 mg per day. As an objective sign that the prostatic enlargement set up certain urination obstruction, these animals exhibited an increase in the weight of the bladder also.

Table 11. *Normal animals.*

Dog	Age in months	Body weight in kg	Height of shoulder in cm	Prostate. Weight in gm per 20 kg body weight	Bladder. Weight in gm per 20 kg body weight
1	17 1/2	20.0	58	7.9	11.8
2	27	24.3	61	17.2	14.1
3	30	23.9	59 1/2	14.3	16.5
4	25 1/2	19.5	57 1/2	24.7	17.7
5	23 1/2	21.1	58	11.7	12.7
	M = 24.7	M = 21.8	M = 58.8	M = 15.2	M = 14.6

Table 12.

Dog	Age in months	Body-weight in kg	Height of shoulder in cm	Treatment		Prostate. Weight in gm per 20 kg body-weight	Bladder. Weight in gm per 20 kg body-weight
				Daily dose of testosterone prop. in mgs	Time in days		
6	27	23.8	58	5	37	20.0	17.5
7	28 1/2	20.2	57	5	37	13.8	11.5
8	30	16.9	57	5	37	15.3	10.5
	M = 28.5	M = 20.3	M = 57.3			M = 17.0	M = 12.8
9	27	22.8	55	25	36	40.6	13.1
10	26	23.5	59	25	36	34.2	13.7
	M = 26.5	M = 23.2	M = 57			M = 37.4	M = 13.4
11	21	20.4	59 1/2	25	118	25.4	19.8
12	16	19.0	56 1/2	25	118	38.9	18.4
13	17 1/2	18.9	54	25	115	43.1	22.3
	M = 18.2	M = 19.4	M = 56.7			M = 35.8	M = 20.2
14	16	10.5	55	125	54	33.8	23.2
15	15	20.6	57	125	54	51.6	19.9
16	15	19.1	55	125	54	36.0	24.4
	M = 15.3	M = 16.7	M = 55.7			M = 40.5	M = 22.5

The probability of the significance of the weight increase was calculated according to FISHER's t-test (1930), on account of the limited number of observations.

On histological examination, the glandular enlargement proved due to a diffuse and on the whole homogeneous hyperplasia (figs. 60—64). The height of the epithelium is raised (figs. 67 and 68); sometimes it fills out the alveoli almost completely. In other cases the alveoli have

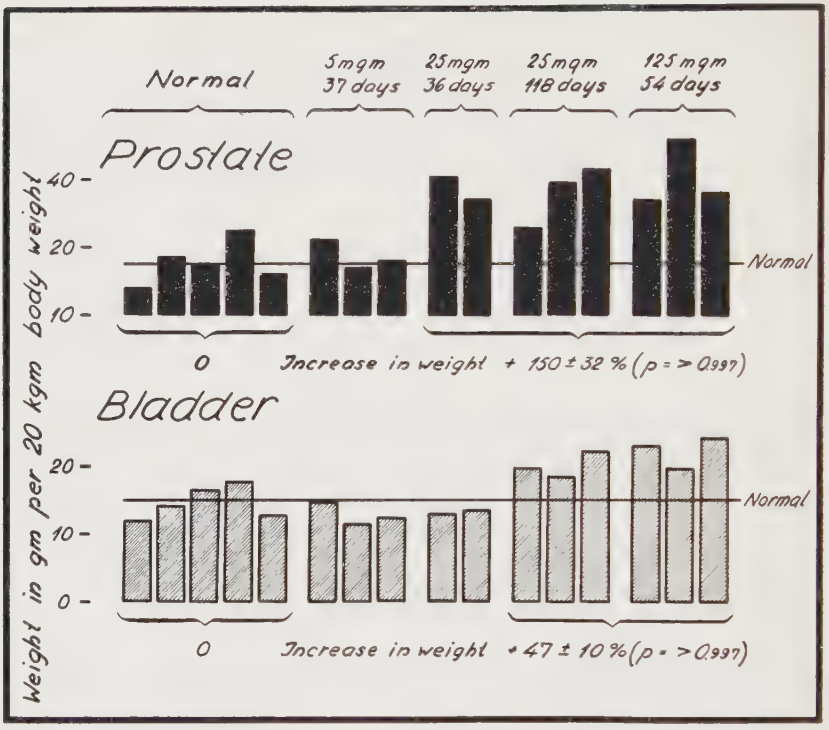


Fig. 50. Weight of prostate and bladder in normal dogs and dogs treated with testosterone propionate. p = probability of significance.

been cystically widened. The hyperplasia of the adjacent glandular parts found in the connective tissue round the urethra is particularly marked (figs. 65 and 66). It resembles the hyperplasias, described by KOTTMAN (1935), of the periurethral glands attendant on spontaneous prostatic hypertrophy in the dog. On the other hand, larger nodules of the type characteristic for the fully developed prostatic hypertrophy in the human subject were not observed. This need not mean, however, that testosterone is unable to produce such changes. Spontaneous prostatic hypertrophy is developed during the course of years or decades. In these tests the time for treatment has had to be considerably shorter.

Attempts have also been made to produce enlargement of the prostate in dogs by inhibiting the internal secretion of the germinal tissue by means of X-ray treatment. In so doing, rather younger dogs were used than in the experiments mentioned above. The weight of the prostate

varied greatly both in normal animals and in those receiving treatment; it was rather larger in the latter than in the former. Nevertheless the difference was negligible, and the probability of significance slight.

Summary. The administration of testosterone propionate to dogs produces an enlargement of the prostate causing obstruction of the urethra. The histological changes of the gland resemble those occurring in spontaneous prostatic hypertrophy in dogs.

SUMMARY.

1) In the testicles, as in the ovaries, two hormones are formed, one in the germinal tissue and one in the interstitial cells. The latter hormone (testosterone) stimulates the growth of prostate and seminal vesicles.

2) The cessation of the germinal tissue's internal secretion, like castration, sets up in rats a weight increase of the hypophysis, as well as an increased production, via the hypophysis, of the testis hormone formed in the interstitial cells which stimulates the growth of prostate and seminal vesicles.

3) The hormone formed in the germinal tissue is characterized in relation to the hormone (testosterone) formed in the interstitial cells by a more potent effect on the weight increase of the hypophysis, occurring in rats after castration, than on the prostate and seminal vesicles.

4) A fraction was isolated from the testis tissue, which when given to young castrate male rats in small doses inhibits the weight increase of the hypophysis which sets in after castration; larger doses intensify it. Such doses as inhibit the weight increase of this organ do not affect that of the prostate and seminal vesicles. The fraction in question thus possesses the qualities given for the germinal tissue hormone. As regards tested biological properties, this fraction has shown itself to tally with estradiol. In respect of fractionization, also, the larger part of the biologically active substance in this fraction similarly tallies with estradiol. No support has been found for the existence in testicular extracts, of non-estrogenic substances, which possess the biological properties outlined for the germinal tissue hormone.

5) By administering estrogen, it is possible to compensate the changes observed in the hypophysis and accessories on the cessation of the internal secretion of the germinal tissue.

6) The administration of testosterone-propionate to dogs sets up enlargement of the prostate with obstruction of the urethra. The histological changes of the gland resemble those appearing in spontaneous hypertrophy of the prostate in dogs.

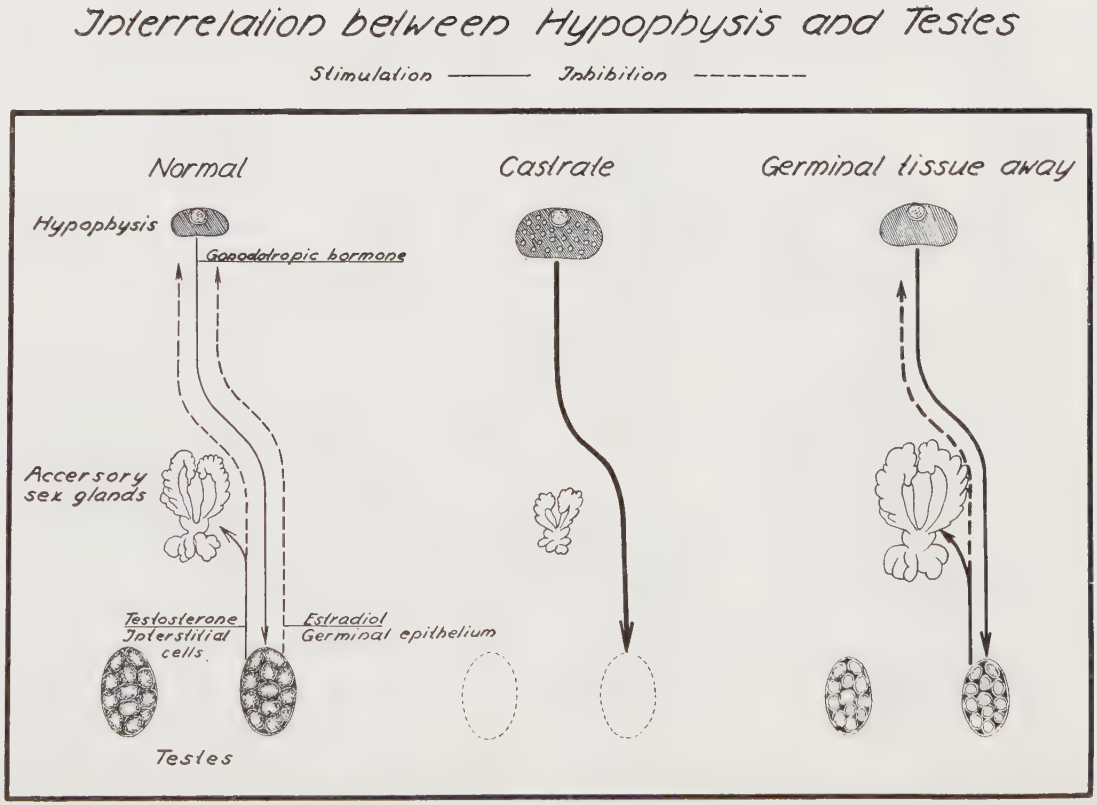

Interrelation between Hypophysis and Testes
Stimulation ——— Inhibition - - - - - - -
Normal
Castrate
Germinal tissue away
Hypophysis
Gonadotropic hormone
Accersory sex glands
Testosterone Interstitial cells
Estradiol Germinal epithelium
Testes

Fig. 51.

7) The interaction between the internal secretions of hypophysis and testicles is shown in fig. 51.

The results are important with regard to the etiology and therapy of human prostatic hypertrophy. An increased action of testosterone induces prostatic hypertrophy in dogs. Lowered internal secretion of the germinal tissue of the testicles sets up, by way of the hypophysis, increased production of testosterone. If hypertrophy of the prostate is caused in the human being by a corresponding change of the internal secretion of the testicles, then it should be possible to forestall this complaint by administering the hormone which is formed in the germinal tissue.

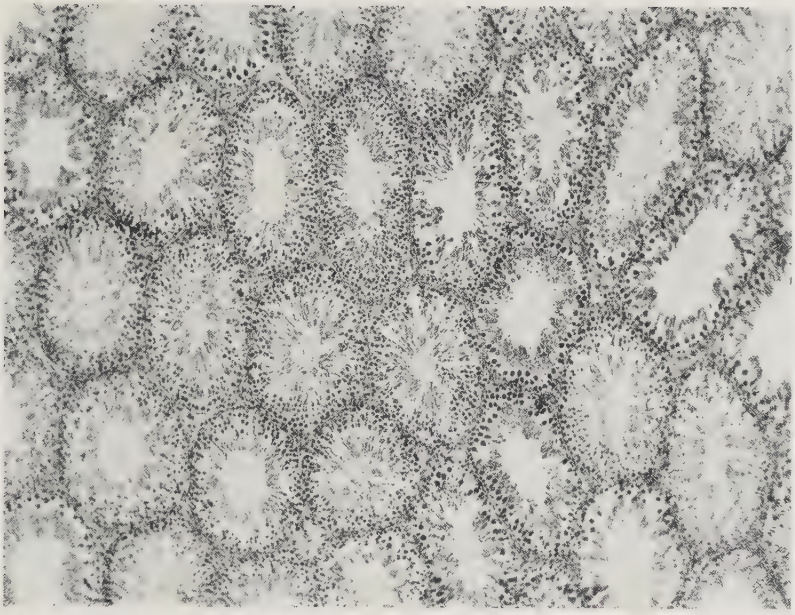


Fig. 52. Testicle of normal rat. Series AI. × 76. Haematoxylin and Eosin.

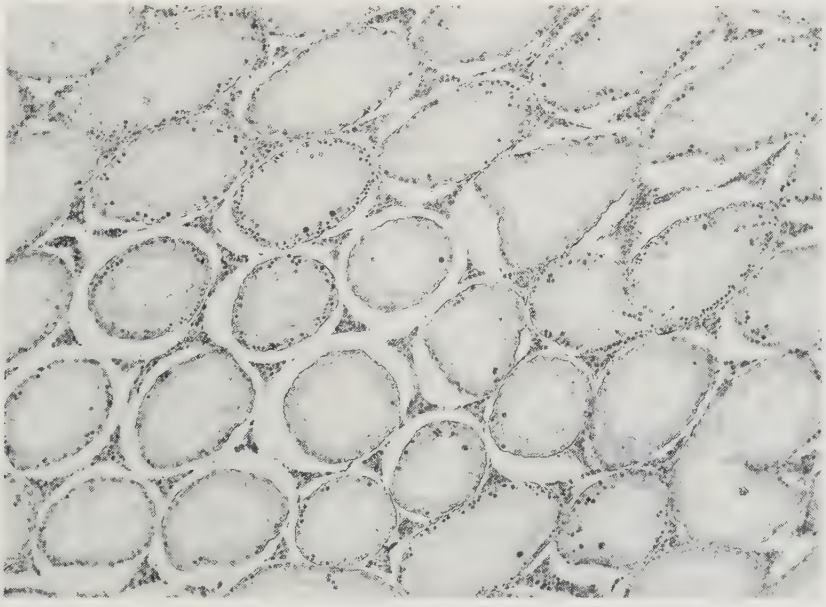


Fig. 53. X-rayed testicle. Series AIXb. Atrophy of the germinal tissue. Interstitial cells well preserved. × 76.

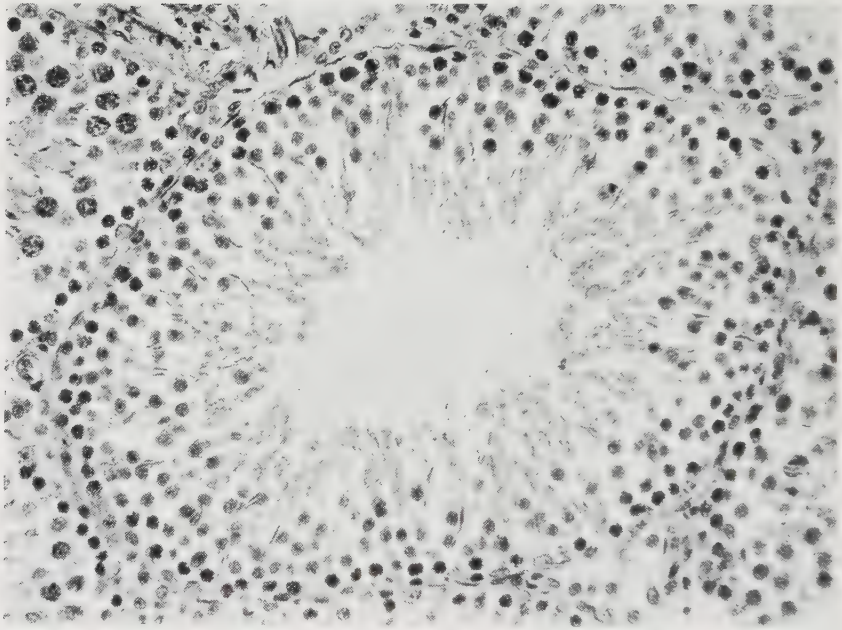


Fig. 54. Testicle of normal rat. Series AI. × 300.

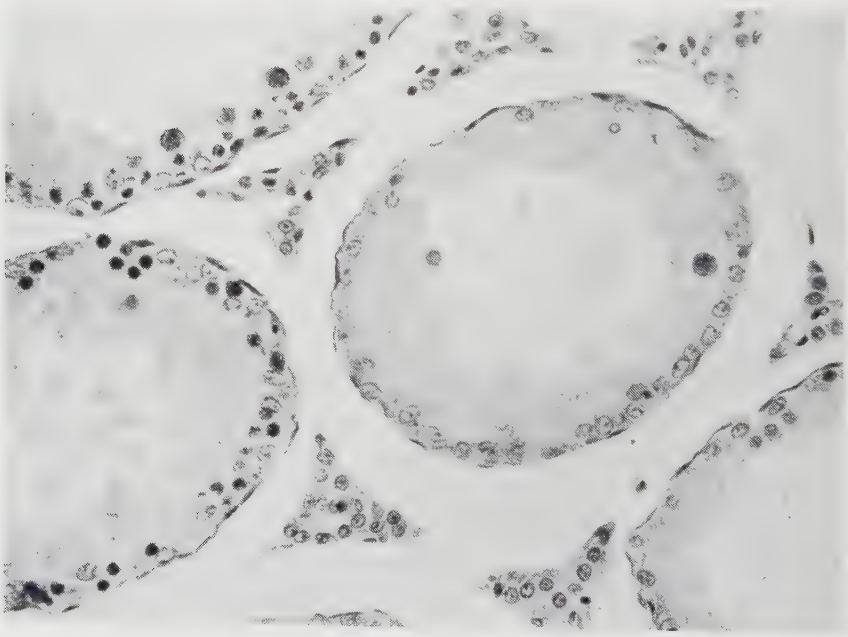


Fig. 55. X-rayed testicle. Series AIXb. Atrophy of the germinal tissue. Interstitial cells well preserved. × 300.

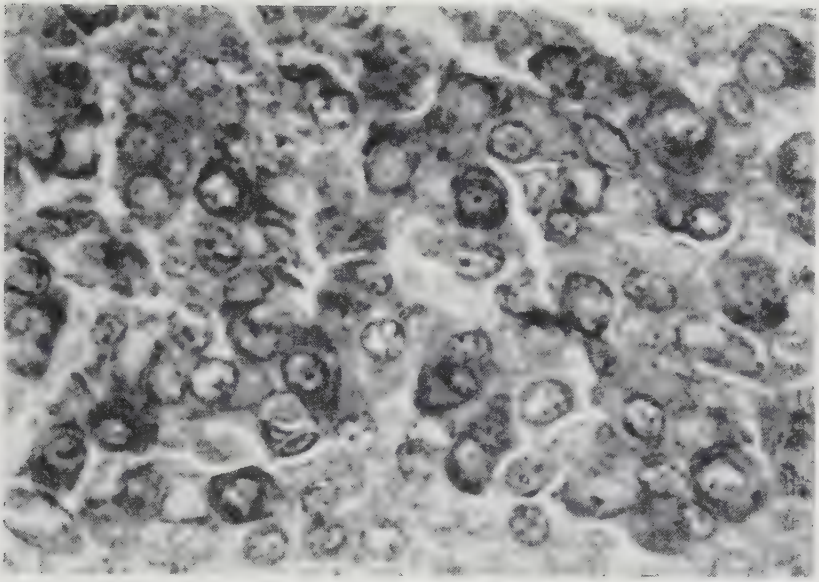


Fig. 56. Hypophysis from normal young male rat. × 1000.
Anilin blue and Orange G.

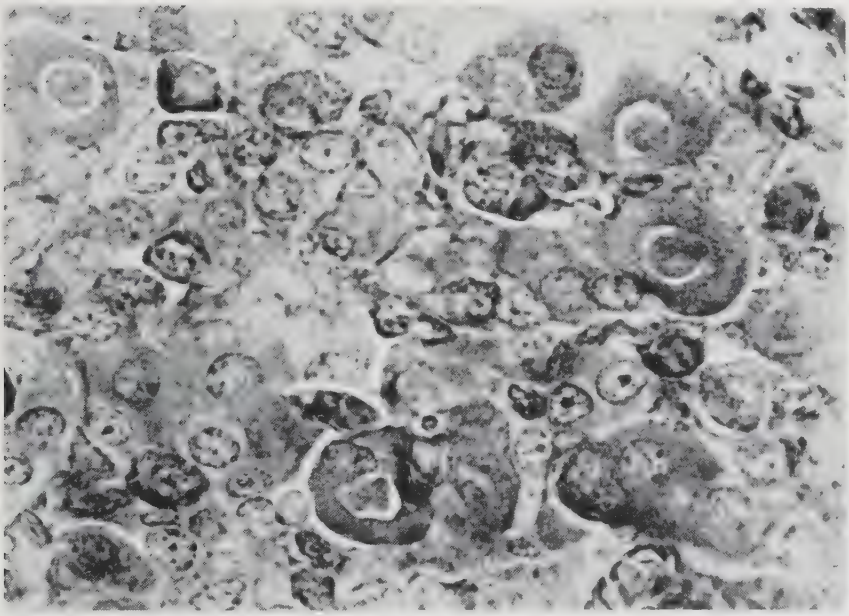


Fig. 57. Hypophysis from young castrate male rat. 3 weeks after castration. × 1000.

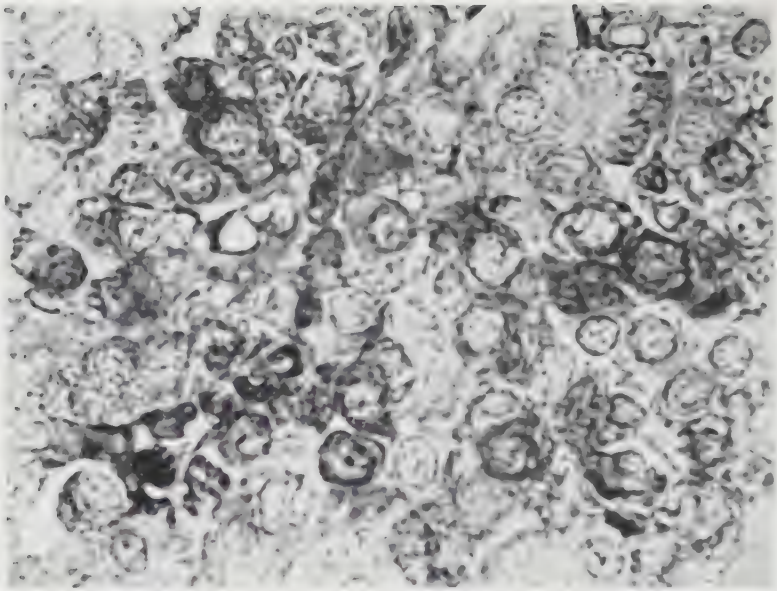


Fig. 58. Hypophysis from young castrate male rat having received 62.5 γ testosterone propionate daily for 3 weeks. × 1000.

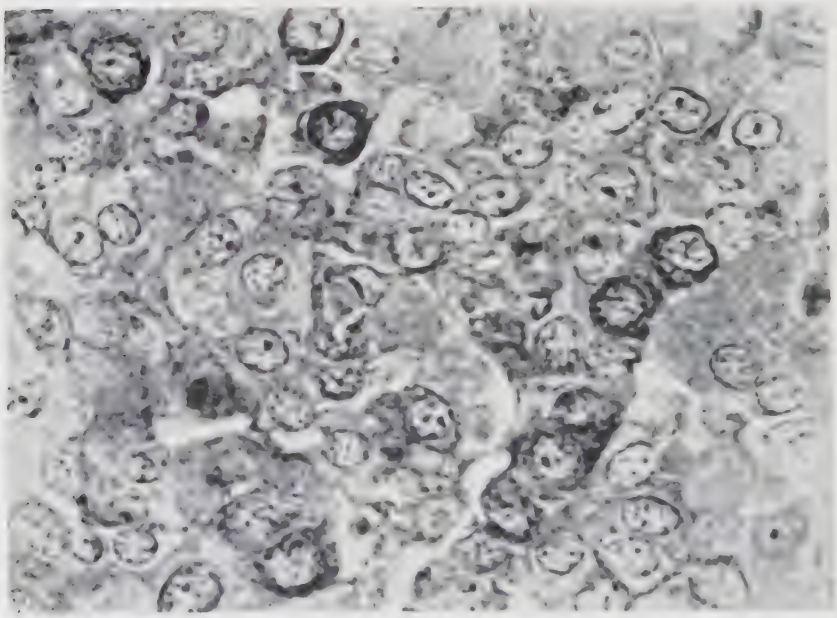


Fig. 59. Hypophysis from young castrate male rat having received 4 MU fract. 2HVIIa daily for 3 weeks.

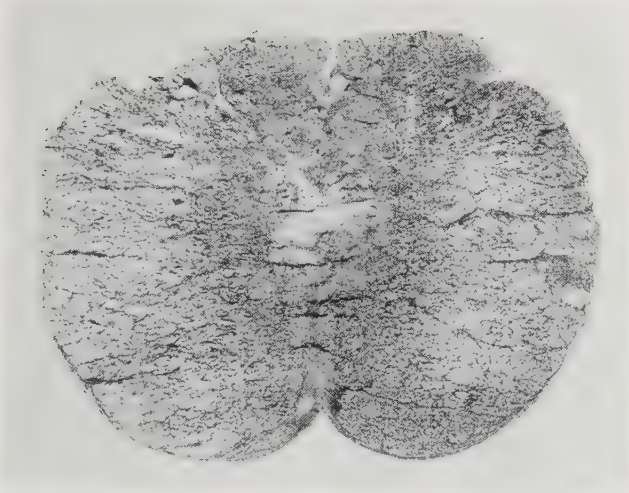


Fig. 60. Cross section of prostate from normal dog. Dog 2. × 2. Haematoxylin and Eosin.

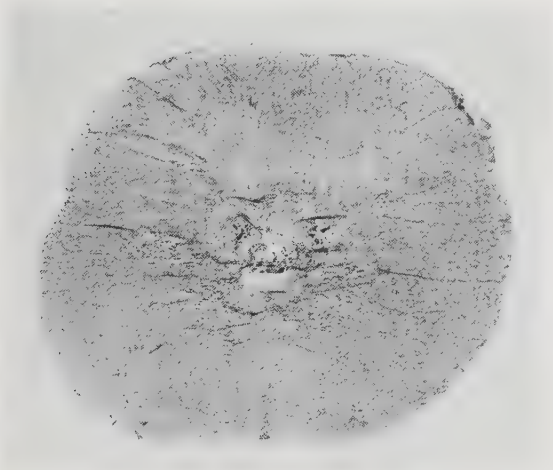


Fig. 61. Cross section of prostate from normal dog. Dog 5. × 2.

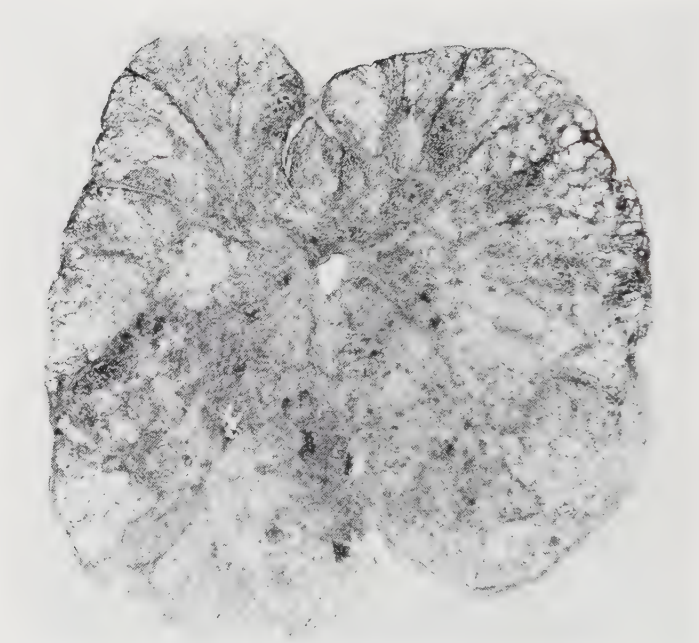


Fig. 62. Cross section of prostate from dog given 25 mgs testosterone propionate daily for 36 days. Dog 9. × 2.

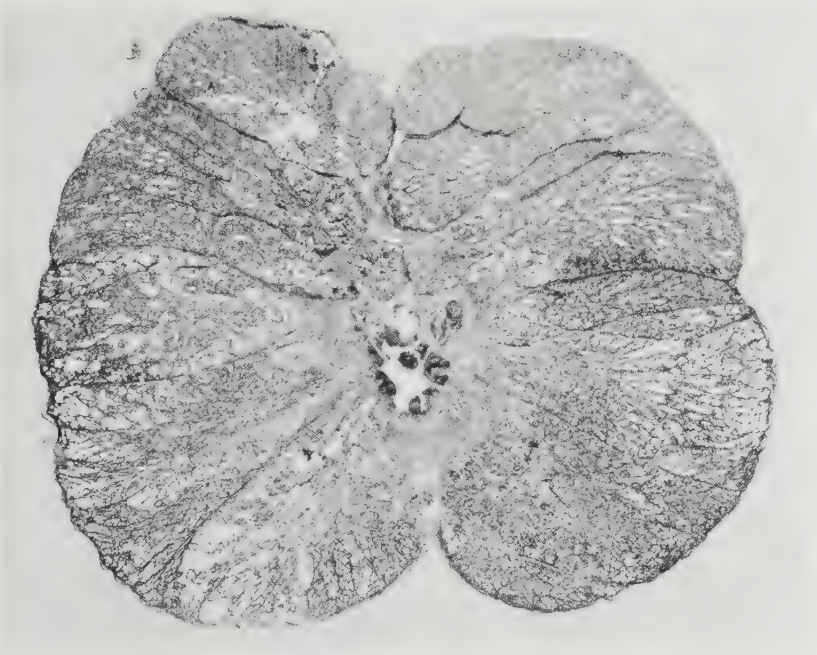


Fig. 63. Cross section of prostate from dog given 125 mgs testosterone propionate daily for 54 days. Dog 15. × 2.

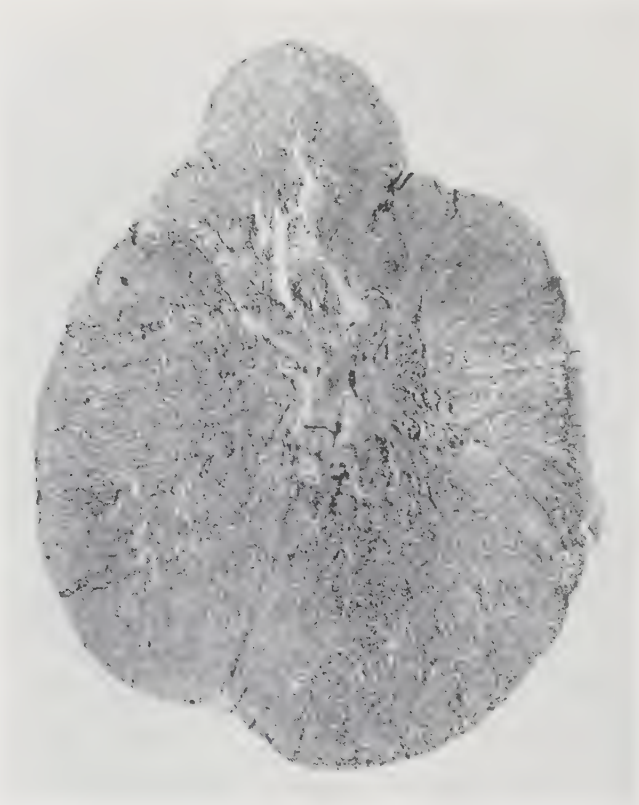


Fig. 64. Cross section of prostate from dog given 125 mgs testosterone propionate daily for 54 days. Dog 16. × 2.

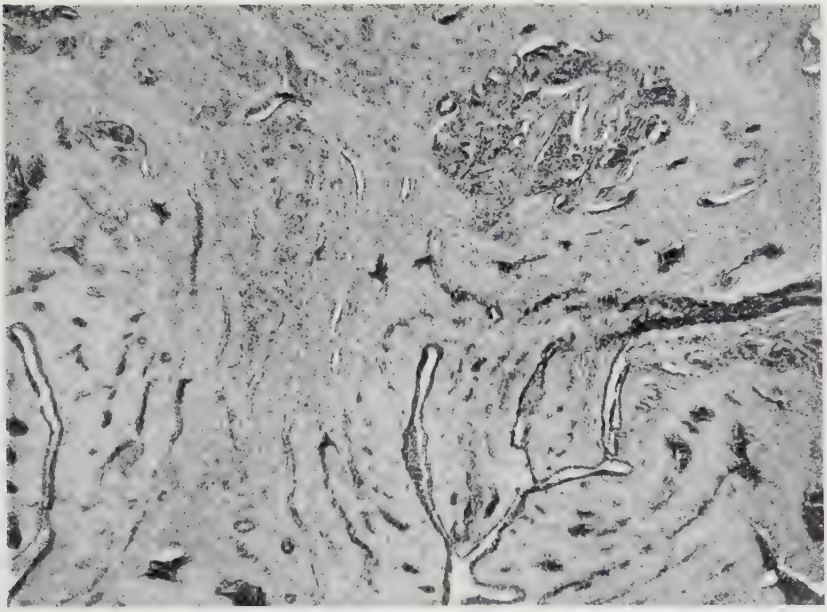


Fig. 65. Prostate from normal dog. Dog 5. In the lower part of the picture a cross section through the urethra. In the upper part periurethral gland. × 44.

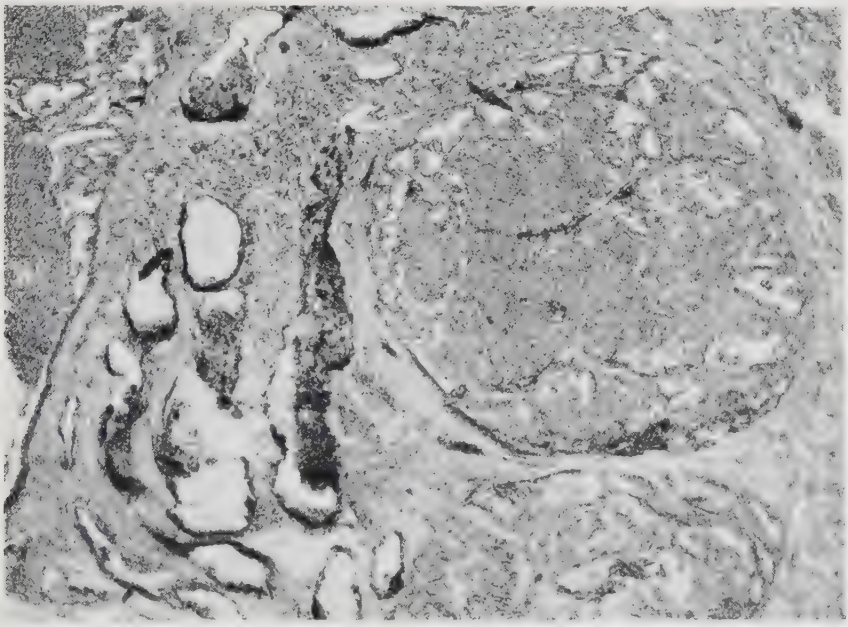


Fig. 66. Prostate from dog given 25 mgs testosterone propionate daily for 118 days. Dog 12. On the left, urethra. Right, hyperplastic periurethral gland. × 44.

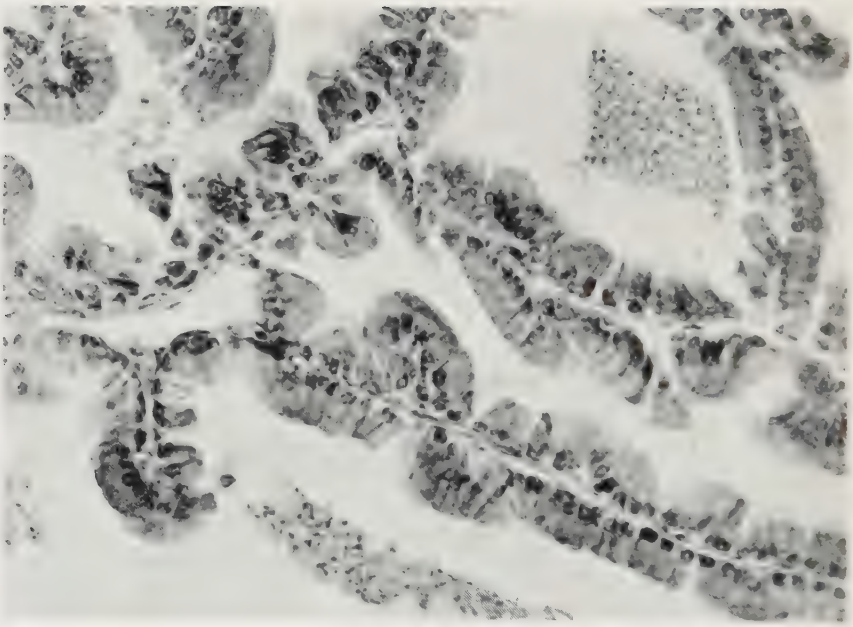


Fig. 67. Prostate. Normal glandular epithelium. Dog 2. × 400.

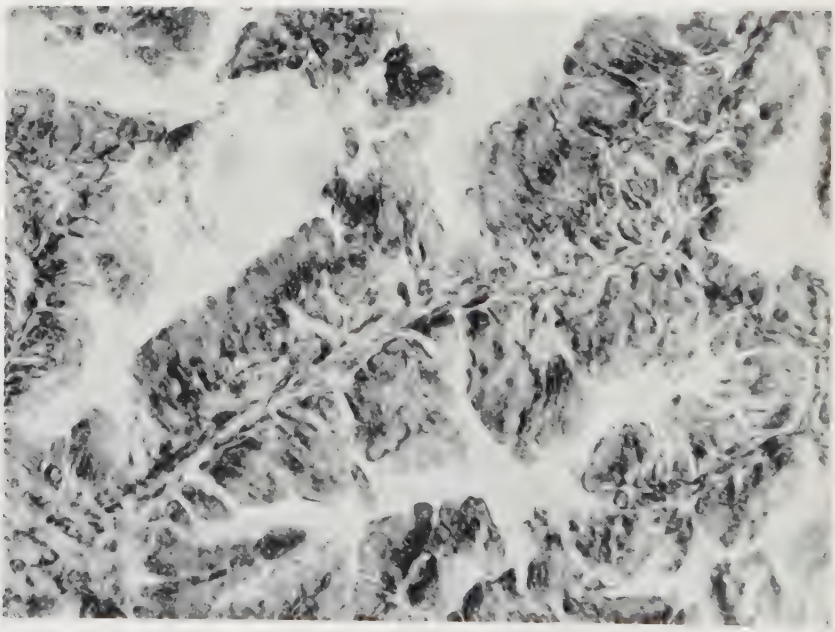


Fig. 68. Prostate from dog given 125 mgs testosterone propionate daily for 54 days. Dog 16. Increased height of glandular epithelium. × 400.

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